CHEMICAL ANALYSIS AND ASSESS OF THE NUTRITIONAL VALUE OF THE SEED OF TETRACAPIDIUM CONOPHORUM

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DECLARATION

I declare that this thesis is my own work and has never been submitted elsewhere in any form for any degree. All information cited from published or unpublished data has been gratefully acknowledged in the text.

Signed: ----------

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Date: 10/11/89

THESIS APPROVAL

This thesis entitled: CHEMICAL ANALYSIS AND ASSESSMENT OF THE NUTRITIONAL VALUE OF THE SEEDS OF TETRACARPIDIUM CONOPHORUM, by ABDULLAU, MUHAMMAD SANI, meets the regulations governing the award of the degree of MASTER OF SCIENCE of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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SALLAU, MUHAMMAD SANI.

DEDICATION

This Thesis is dedicated to my parents SALLAU IBN ABUBAKAR SADIQ and MAIMUNAT BINT MUHAMMAD and for the evalasting memory of my Grand Mother HAUWA'U MUHAMMAD.

ABSTRACT

The evaluation of the nutritional value of the seeds of Tetracarpidium conophorum (Mull, Arg,) Hutch & Dalza, which belongs to the family of Euphorbiaceae, was carried out on four different samples which include the raw, the cooked, the raw cotyledon and the raw cotyledon-removed kernels of the seed.

The analysis shows that the raw seed kernel contains 35.49% moisture (at 60 C), 40.30% moisture (at 105 C) both of which were on fresh weight basis. The dry raw sample contains 40.3% ash, 95.54% total organic matter, 49.16% crude fat, 20,62% crude protein (Kjeldahls method), 21.06% crude protein (Nesslers reagent method), 25.76% total carbohydrate, 5.78% soluble carbohydrate and a calorific value of 627.96 kilocalories per 100g of dry sample. The amino acid composition showed some of the essential amino acids like isoleucine, phenylalanine plus tyrosine and methionine to be above the values recommended by FAOWHO (1973). Tryptophan was destroyed on hydrolysis and threonine was no detected.

The mineral elements determined in the raw seed sample were Cd: 2..26 ugg^{-1}, Ca: 0.725%c, Cu: 20.11 Ugg , Fe: 0.030%, Ng: 0.106%, Mn: 0.010%, P: 0.86%, K: 1.6)4%, Nai 0.076% and Zn: 62.17 ugg^{-1} Cobalt was however not detected at the working concentration range employed.

Cooking was found to have led to the destruction of Valine and a decrease in the concentration of other amino acids detected (with the exception of aspartic acid). It has also decreased the crude fat, the soluble carbohydrate and the total calorific value in addition to a decrease in the concentration of a number of essential nutrient elements. In particular, it decreased the concentrations of a number of

antinutritional elements by increasing the potassium to sodium and zinc to cadmium ratios from 21.58 to 33.78 and from 21.50 to 74.25 respectively.

The cotyledon sample has proven to have a higher concentration of most of the nutrients than the other samples analysed. These findings were discussed in terms of the value of the nut as food for man and animals.

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CHAPTER ONE

INTRODUCTION

1.1 Justification for the Work

The evaluation of the nutritional value of plant food materials is of paramount importance because of the role they play as major constituents of the necessary foods required for growth and survival by living organisms. MacCance and Widdowson (1969) reported that the nutritional and dietetic treatment of disease as well as research into problems of human nutrition demand an exact knowledge of the chemical composition of foods.

Nigerian scientist, like those of other developing countries are conducting research in almost all fields of human endeavour and as usual use laboratories to achieve most of their objectives. Nutrition is one of the areas of research in which rats and other animals are used as samples. This is due to the fact that inadequate and unbalanced nutrition is one of the main obstacles of livestock production and has been a problem in human nutrition (Ega, 1986). Scientific and economic feeding experiments however are based on the provision of a balanced ration, by which the animal is supplied daily with the correct amounts of the various feeding constituents, necessary to maintain daily growth and vigour (Wood and Woodman, 1939). The compounding of a balanced ration or diet, necessitates a knowledge of the composition of the various foodstuff employed, and the principles on which the calculation is based (Georgi, 1934). It is therefore in recognition of the potential use of the seed samples analysed in the present work, for various feeding and nutritional purposes that the researcher aims at discovering the chemical composition of the sample specie.

Moreover, in consideration of the vigour with which the Federal Government of Nigeria is pursuing the attainment of self-sufficiency in food production, emphasis needs to be given to such an analysis that can furnish informations as to which specie of plant food materials are of greatest nutritive significance to the people. Such a knowledge will indeed assist the Authorities in marking out which particular plant food materials need to be given special consideration in such a drive as to provide the overwhelming population with sufficient dietary food materials.

It has recently been understood that plant materials which are regarded to be of second class quality are found to be deficient in one essential nutrient or the other (Haikewal, 1969). It is however worth mentioning that a suitable mixture of vegetable food materials may result in foods with first class quality. For instance the lack of an essential amino acid in one plant food material is made up by ingestion at the same meal of the other foods supplying the missing amino acid (Ega, 1986).

Quite a number of plant food materials consumed by a population of a community are not nutritionally useful because of the amount of toxicants occuring naturally or articially in them. The presence of such toxicants could pose great hazards to the health and well-being of the consumers. For instance, George (1973) reported that there is an incontrovertible evidence that excess sodium raises blood pressure in some humans, and that extra potassium is beneficial in some hypertensives. It is therefore only through the chemical and biochemical analysis that an evaluation could be made of the nutritive quality of any one food material, especiall as regards its composition of both nutritive and toxic components.

Considering the multifarious nature in which the present seed samples are consumed, an evaluation needs to be made of the advantage or otherwise of cooking the seeds prior to consumption and whether one component of the seed kernel is more nutritionally useful than the other. In addition, the claim that the leaves of the plant bearing the seeds are useful medicinally (Dalziel, 1955) is a valuable information for further analysis of the sample.

Proteins from animals such as birds and fish, milk, egg and those from plants like the legumes such as groundnut, peas and beans are expensive but heavily consumed by humans. Therefore, the seeds and fruits from many trees which are sparingly eaten by humans are often not exploited and need to be investigated. Thorough research is needed to evaluate the possibility of using these fruits and seeds as protein concentrates in livestock feeding as well as in human food (Ega, 1986).

These and many other factors prompted the researcher of the present work to aim at:

(a) developing a comprehensive data bank on the chemical composition of the analyte sample.

(b) finding out the chemical composition of the sample needing further investigations.

1.2 Types of Samples Analysed

The plant, Tetracarpidium conophorum (Mull., Arg.) Hutch. & Dalza, otherwise known as Asala and whose seeds are to be analysed is a dicotyledonous angiosperm, belonging to the family of Euphobiaceae (Figure 1. It is commonly called "awusa" (Yoruba), "Okhue" or "Okwe" (Benin) and Ibuzo "akan otoli" (Ibo), (Dalziel, 1955). The Hausas in Northern

Family Euphorbiaceae

Name Tetracarpidium conophorum (Mull.Arg.) Hutch.& Dalz.

Det. A.O. Chaeri

Locality Chokobe Ndume Ibeku, Imo State

Habitat Bush near village

Description

Climber, flower greenish yellow. Fruit with three or four chambers. Seed edible.

Coll. A.O. Chaeri

Coll. No. 2144 Date: 16/12/82

Fig. 1.1 A preserved specimen of Tetracarpidium conophorum

Nigeria call it "Gyadar Yarabawa", meaning "Yoruba groundnut" because it is brought to the North from the Yoruba part of the country and testes just like groundnut when eaten cooked.

The plant was first reported from Sierra Leone, Dahomey and B. Congo. Just then, cultivation trials, which were later reported to be successful were carried out in Nigeria. It is a woody liane, up to 100 ft tall in low bush; leaves are globrous, ovate long and the margins toothed. The base is broad and rounded up to 5x3 inch and with slender petioles up to two inch long and three nerved at base. The male flowers are greenish in narrow receme-like inflorescences up to five inch long with one to two females near base. The pedicels are joined above base, stamens numerous, style stout becoming quadrangular and stigmas four. The fruit is four winged ridged between wings and up to three inch diameter. The seeds are subglobose and about one inch long (Irvine, 1961).

The plant is sometimes cultivated in parts of West Africa for its edible nuts, especially on recently cleared land. It starts fruiting in its second year. The seeds which are sold in markets are cooked, and often eaten with maize. The fruit contains subglobose seeds with a hard thin brown shell and yellowish kernel (Dalziel, 1955).

The fruit yields 48-60% of a golden yellow rapidly drying oil, possibly suitable for edible purposes, for soap making and as a substitute for linseed oil in the varnish and lacquer industries (Irvine, 1961). For instance, the kernels of fruits obtained from Cameroon yield up to 59% of a rapidly drying oil, whitish yellow with agreeable taste and odour, having high iodine index and with physical and chemical properties similar to linseed oil (Dalziel, 1955).

The seeds are eaten like Walnuts and in the lower Congo region, the people regard them as having the same properties as Kola, the local

product being somewhat bitter. In Southern Nigeria the leaves of the plant are used to "wash children so as to cause other babies to be born". The leaves are applied medicinally to treat headache (Dalziel, 1955

Despite the fact that some parts of the plant fruit are edible, the present analysis is selectively carried on the edible portion of the plant seed; the kernel, after removing the hard shell cover. The seed kernel is chosen because it is reported to be the most widely consumed (Irvine, 1961). Each fruit however can contain up to four seeds, in addition to the fruit juice.

1.2.1 Source of Fruit Seed Analysed

Fresh samples for analysis were obtained from Zaria Railway Station. They were fresh, just arriving directly from the south western part of the country. This measure was taken because of the report that the seeds do not store well unless immediately heated to 100°C (Irvine, 1961).

The hard shell was broken and the seed kernel removed. All the sample analysis was therefore done on the seed kernel. Earlier, Irvine (1961) reported that the seed kernels are eaten raw or after the seeds are cooked. Analysing the cooked sample was then necessitated by the report that reduction in the amounts of certain amino acids can be caused by the process of cooking, although actual destruction seldome occurs (McCance and Widdowson, 1969). This prompted the analysis of the cooked sample so as to assess the extent of the reduction in the amounts of certain amino acids as a result of cooking.

1.3 Scope of Analysis

The efforts made to evaluate the nutritional value of the sample specie are mainly to determine its chemical and physical scores. The

chemical score refers to all the chemical analysis carried out on the food sample while the physical score refers to the evaluation of its nutritional value by its energy content based on the results obtained from the chemical analysis of the food sample (McCance and Widdowson, 1969).

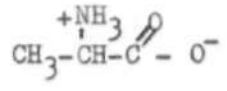
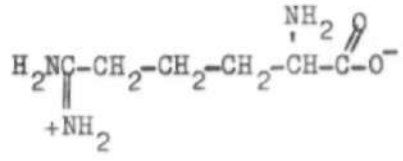
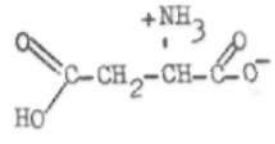
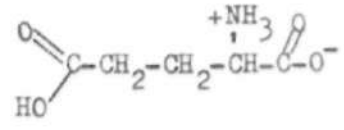
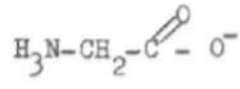
The chemical analysis done comprised of the determination of the proximate constituents, mainly the moisture contents, ash and organic matter contents, Crude Fat (moisture free ether extractable portion), crude protein and Total and soluble carbohydrate content of the food material. Other items considered in the chemical analysis include the mineral content and the amino acid composition.

The moisture content was determined at two temperatures of 60°C and 105°C. The crude protein content was determined by two methods of classical kjeldahl's distillation method and the colorimetric method of Nesslers reagent, so that a comparison could be made. The elements considered under the mineral content determination include Sodium, Potassium, Calcium, Magnessium, Manganese, Iron, Zinc, Phosphorus, Copper cadmium and cobalt.

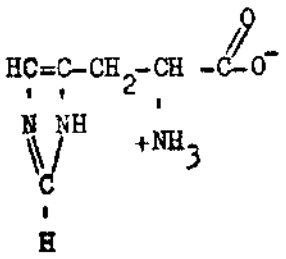
The amino acid analysis of the sample is prompted by the fact that the functional, physical and chemical properties of a protein depend on the amino acid constituents which occur in the peptide chain (Haikewal, 1969). The nutritive value of proteins therefore is not necessarily determined by its quantity, but by its quality which refers to the amounts and types of amino acids it has. Although there are more than 200 amino acids that occur in nature particularly in the plant kingdom, most do not appear in proteins (Baum, 1978). There are presently only about 26 commonly occuring amino acids in proteins (Oni, 1979). The protein amino acids include both essential (indispensable) and the non-

essential (dispensable) amino acids. For instance there are nine indispensable amino acids for man (Histidine, methionine, tryptophan, threonine, leucine, isoleucine, valine, phenylalanine and lysine). Histidine is indispensable for human infant and cystine and tyrosine are semi-indispensable since they can be synthesised in the body from methionine and phenylalanine respectively (Harper, 1973).

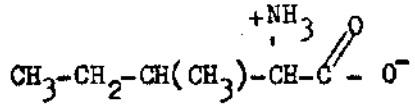
Of all the protein amino acids mentioned, only sixteen have been investigated in this work because of the method of acid hydrolysis employed. In this method, only sixteen of the eighteen common amino acids are liberated. This is because Tryptophan is completely destroyed and cysteine and cystine are partially destroyed (Haikewal, 1969). Those investigated using the Technicon sequential Multisample amino acid analyser, located in the Nutrition research laboratory of Biochemistry Department, Ahmadu Bello University, Zaria include the following:-

	Amino Acid	Dipolar ion Structure
(1)	Alanine	
(2)	Arginine	
(3)	Aspartic acid	
(4)	Glutamic acid	
(5)	Glycine	

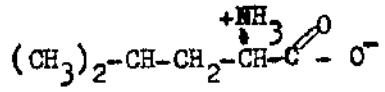
(6) Histidine



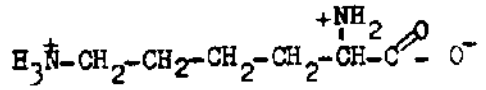
(7) Isoleucine



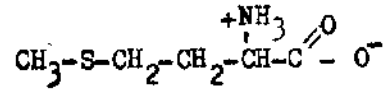
(8) Leucine



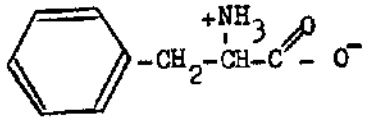
(9) Lysine



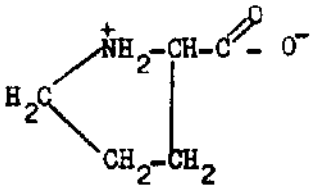
(10) Methionine



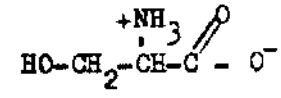
(11) Phenylalanine



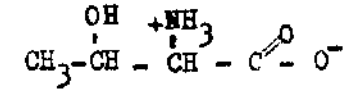
(12) Proline



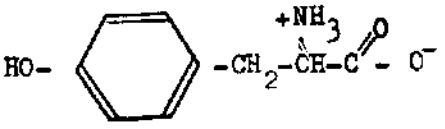
(13) Serine



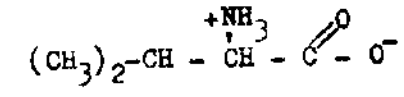
(14) Threonine



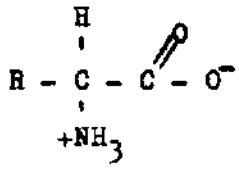
(15) Tyrosine



(16) Valine



The dipolar ion structure of amino acids play a significant role in their quantitative estimation by means of ion exchange chromatographic methods of amino acid analysis.



The ion exchange methods of amino acid analysis depend on the differential binding of charged groups on to the supporting medium which is made up of highly cross-linked high molecular weight polystyrene polymers carrying ionisable groups. The degree of separation is related to the respective PKa values of each amino acids. In addition, aliphatic and aromatic amino acids are adsorbed to varying degrees on the supporting medium and this property plays an important role in the separation (Haikewal, 1969).

On the other hand, the determination of the physical score was accomplished by the evaluation of the energy content. This was achieved by using the results obtained from the chemical score by using some appropriate conversion factors called Atwater factors (Pearson, 1970). These factors, obtained by combustion or burning the foodstuff in the presence of oxygen using a bomb adiabatic calorimeter, signify that burning 1g each of carbohydrate, protein and fat yields 4, 4 and 9 kilocalories of energy respectively (Ranjhan and Krishna, 1980).

CHAPTER TWO

REVIEW OF LITERATURE

2.1 Proximate Constituents of Seeds and Nuts

Most of the work reported so far on the nutritional studies of plant seeds and nuts and other plant food materials in general is confined to their proximate composition (McCance and Widdowson, 1969). This is probably because the plant food materials are generally classified to be of second class quality as against their animal counterparts. Nevertheless quite a large number of different plant species have been analysed.

The routine analysis of foods is termed the proximate or Weende analysis, named after the Weende experimental station, in Germany, developed in 1965 (Oyeleke, 1984). The proximate analysis which comprises the determination of Moisture content, Ash and Organic matter content, Crude fats and Protein, Total and Solube (available) carbohydrate and the Mineral composition of the plant food materials, always furnishes the basic informations necessary for an assessment of the quality of the particular food material required. A lot of research on the analysis of seeds has therefore gone in this direction so as to achieve the said objectives. For instance, Eka (1971) analysed the chemical composition and the uses of kolanuts and Itam *et al* (1983) reported the chemical evaluation of the nutritive value of African Walnut - *Coula edulis* (Baill). Kapu (1980) also determined the protein and mineral values of seeds and fruits of some Nigerian Trees and McCance and Widdowson (1969) published the composition of various foods in which the composition of seeds and nuts has been included. Moreover, Pearson (1970) also reported the compositional data of the kernels of various nuts.

It is in this regard that the present sample of analysis is chosen because there is no current publication on a similar analysis of the seed specie.

2.1.1 Moisture Content

The determination of moisture content of foodstuff is of imperative importance especially for storage purposes. The safe limit reported is 15% moisture because in cases where the value exceeds this limit, food materials should not be stored as they may develop the undesirable moulds and fungi (Ranjhan and Krisha, 1980). For instance moulds may grow when the moisture content of groundnut exceeds 9% and 16% in the case of the groundnut meal (Pearson, 1970).

Moreover, grains that contain too much water are subject to rapid deterioration from mould growth, heating, insect damage and sprouting. A knowledge of moisture content is needed for optimum processing of foods, for example, milling of cereals, mixing of dough to optimum consistency and for producing bread with the best grain, texture and freshness retention (Pomeranz and Meloan, 1978). In addition, the presence of water in powders causes caking for example for sugar and salt (Pearson, 1973).

Due to the importance attached to moisture content determination, quite a lot of work has been carried out in this direction on a number of plant fruit and seed species. Both McCance and Widdowson (1969) and Pearson (1970) reported that the kernels of Almonds sweet, Brazilnuts, Peanuts and Walnuts have a water content of 4.70%, 8.50%, 4.50% and 23.50% respectively. However, no such similar work has yet been reported on the present sample for analysis.

2.1.2 Seed Proteins

The word "protein" which first appeared in scientific literature in 1838 in an article by one Dutch chemist G.L. Mulder, is derived from a Greek word "Proteu" meaning "I occupy first" (Haikewal, 1969). The investigations on seed protein began much earlier by Beccare in 1745 when he separated gluten from wheat flour and although marked advances were made by other researchers, the first serious and long study on proteins came from Rittansen in 1962 whose work was carried on by Osborne in 1891.

Haikewal (1969) reported that the plant angiosperms are botanically divided into two main groups, the monocotyledons and the dicotyledons. The former have seeds with a single storage organ, the endosperm, and the embryo lies close to it at the end where the grain is attached to the ear. The dicotyledons however store their food in two cotyledons and the endosperm which surrounds the whole embryo. It is in this regard that proteins from many dicotyledonous seeds are considered practically as embryo proteins, the endosperm being almost absent.

The need to satisfy the nutritional requirements of a significant part of the world population namely, the developing nations cannot be over emphasised. In most cases this nutritional insufficiency is attributed to non-availability of proteins. Since the lack of economic source of animal protein is acute, vegetable or plant protein is increasingly being resorted to, inspite of the fact that the best quality proteins come from animal sources (Sikka et al, 1979). This is because it is possible to formulate satisfactory diets to meet the requirements for both man and livestock from appropriate plant mixtures (Ega, 1986). For instance, corn protein is low in lysine while soya-bean protein is high in lysine. As such if the correct amounts of

soyabean and corn are mixed together, all the essential amino acids required by either pig or rat may be provided without further supplementation (Maynard et al, 1979).

This kind of experimentation has been done successfully in several places for instance Soyogi mixture produced by the Federal Institute for Industrial Research, Oshodi, Lagos, Nigeria (Ega, 1986). Soyogi is a food mixture consisting of corn and soyabeans and has been found to meet the requirements of children. The protein daily requirements for human male adult (70kg), female adult (58kg) have earlier been reported to be 40g, 32g and 12g respectively (Harper, 1973).

2.1.3 Carbohydrates as Energy Sources

Carbohydrates are the most abundantly and widely distributed of the nutrient components in foods. Moreover, it has earlier been reported that carbohydrates are the primary metabolites of the animal kingdom in that over half of the food that the animals consume is composed of this food nutrient (Baum, 1978). In addition to their nutritional and metabolic functions, carbohydrates are important as natural sweetners, raw materials of various fermentation products including alcohols, beverages and the main ingredient of cereals (Pomeranz and Meloan, 1978). Pearson (1970) reported a considerable quantity of Total carbohydrates in Almonds, Peanuts and particularly Cashew. He attributed the sweetness in some nuts to be largely due to the higher amounts of sugars present. Pomeranz and Meloan (1978) on the other hand reported that nuts are generally a poor source of mono- and disarcharides, although chestnuts contain up to 33% starch. They also reported that reducing sugars are the main soluble carbohydrates of most fruits and account for 70% of the seedless raisins.

Carbohydrates, known to serve as fuel of biological systems, supplying living cells with usable energy, are reported to be susceptible to a number of metabolic changes caused by some micro organisms (McGilvery, 1979). Incidentally some of these metabolic changes do occur either in the presence or absence of molecular oxygen. In particular the overall anaerobic fuel metabolism of micro-organism is a process known as fermentation (McGilvery 1979). Carbohydrates of seeds and nuts, just like those of other sources therefore, could be liable to fermentation processes under optimal conditions. Despite the earlier report by Pomeranz and Meloan (1978), that nuts are a poor source of mono- and disaccharides, a lot of research on the fermentation of seeds and grains has already been reported.

For instance, Ega (1986) reported the variations, in nutrient composition, of fermenting the seeds of Parkia clappertoniana (key) and Tamarindus indica (Linn-Holl). She reaffirmed the earlier report by Fetuga et al, (1974), Eka (1980 and 1982) that removal of seed testa, followed by cooking and fermentation definitely improved the nutrient contents of seeds. And although fermentation tended to lower the calorific value, this may not be considered a limitation of the fermentation process since fermented products are usually applied to increase the protein quality and quantity of Carbohydrate and energy-rich diets (Akinrele et al, 1970).

2.1.4 Fats and Oils in Seeds and Nuts

Fat (lipid) is found to constitute a high source of energy since 1g of fat when fully oxidised yields 37.5KJ of energy as compared with figures of 29.8KJ, 17.0KJ and 16.0KJ of energy yielded by equal amounts of alcohol (ethanol), protein and carbohydrate respectively (Oyeleke,

1984). Fats are also needed especially by growing animals, hence it is important to determine their contents in foodstuffs.

Most of the works done so far on nuts and nuts products reveal a very high fat content in the food materials. Both McCance and Widdowson (1969) and Pearson (1970) reported the figures of 53.50%, 61.50%, 49.00% and 51.50% fat contents of Almonds, Brazilnuts, Peanuts and Walnuts kernels respectively. Irvine (1961) on the other hand reported that the fruit of Tetracarpidium conophorum yields 48-60% of a golden-yellow rapidly drying oil which is possibly suitable for edible purposes and as a substitute for linseed oil in the varnish and Lacquer Industries. As the resultant oil is volatile, one appropriate way of its quantitative extraction is by the distillation method for the determination of volatile oils as reported by Pearson (1973).

It is in this regard that an analysis of the fat content of the analyte sample is designed to be made so as to assess the quantity and quality of the fat present.

2.2 Mineral Contents of Seeds and Nuts

Several elements are essential nutrients to both plants and animals. They often play an indispensable role in one metabolic reaction or the other and often form the skeletal framework of many Organic and Inorganic complexes necessary for the smooth functioning of the living system (Allen, 1974).

A number of nutrient elements have been reported in high concentrations in certain plant food materials. For instance, calcium is present in relatively high concentrations in most dairy or dairy containing products; in cereals, nuts, some fishes, eggs and certain vegetables. Phosphorus-rich foods also include most dairy products,

grains and grain products, nuts, meat, fish, poultry eggs and legumes. Other elements found to be present in high concentrations specifically in nuts are Iron, potassium and magnesium (Pomeranz and Meloan, 1978). Another information of nutritive significance is the fact that the ratio of zinc to cadmium in most nuts is reported to be 684 (Schroeder, 1973) an information which implies that nuts are excellent food materials for hypertensive patients. This is because cadmium accumulates in both human and rat kidneys, arteries and liver where it interferes with certain enzyme systems requiring zinc and has more of an affinity for cetain kidney tissues than does zinc, therefore displacing zinc and changing zinc-dependent reactions (Schroeder, 1973). These and many other properties of cadmium make it a causative agent for hypertension in human being (Schroeder, 1973).

Earlier a number of researchers have published the mineral content of the samples analysed. Itam, et al, (1983) reported the elemental composition of African Walnut and McCance and Widdowson (1969) published the mineral content of a number of plant food materials in which nuts were included. Oni (1979) also reported the mineral content of three fish samples he obtained from Zaria dam. These and many other reasons prompted the researcher to undertake an analysis of the mineral composition of the samples under study for a proper evaluation of the nutritive properties of the said samples.

2.3 Amino Acid Composition of Seeds and Nuts

Quite a lot at an advanced stage has been achieved on the amino acid analysis of a number of plant food materials including seeds and nuts. This achievement is certainly not unconnected with the development of Automated Ion exchange methods of chromatographic analysis using a number of instruments. An example is the Technicon sequential,

Multisample amino acid analyser, employed for the present analysis. These instruments facilitate the quantitative and qualitative estimation of amino acids in a very short time.

A number of researchers in the field of food nutrition have reported quite a lot of information on the amino acid composition of food samples. Haikewal (1969) reported the amino acid composition of sorghum grains. McCance and Widdowson (1969) reported the amino acid composition of a number of nuts as produced in Appendix 1. They reported the presence of about eighteen amino acids in peanuts and Fetuga et al, (1973) also reported the same number of amino acids in peanuts meal. On the other hand, Oni (1979) reported sixteen amino acids in three fish samples obtained from Zaria dam. Just recently, Ega (1986) reported the amino acid composition of cassava, cocoyam, the seeds of African locust bean and Tamarind (Appendix 1). She concluded by saying that both tubers contain lower amounts of Arginine than the seeds. Earlier Itam et al, (1983) reported the amino acid composition of African Walnut Coula edulis as reported also in Appendix 1.

Generally however, a review made by Haikewal (1969) of amino acid composition of some seed proteins shows some characteristic differences between dicotyledonous and monocotyledonous seed proteins. For instance, proline content is higher and Arginine content lower in the proteins of monocotyledonous seeds than in the proteins of dicotyledonous seeds. Estimates of the amount of each of the indispensable amino acids require by human adults and children was reported by Harper (1973) and is given in Appendix 2. Of all the work reported on the amino acid composition of seeds and nuts, none has yet been specifically done and reported on the present sample of analysis. It is thus a privilege to subject the present sample specie to an analysis of its amino acid composition.

2.4 Calorific Value of Seeds and Nuts

The process of measuring the amount of heat generated by the combustion of a substance is known as Calorimetry. The energy value of a food is measured in Calories which are physical Units of heat. The number of calories the body can derive from a food is however less than the number of calories produced when the food is burnt in a calorimeter because the calorie-producing nutrients which are mainly protein, Fat and Carbohydrate are not completely digested when eaten, also the products of digestion are not completely absorbed in the human gut and the portion of the protein which is digested and absorbed is not completely oxidised to yield energy in the body (McCance and Widdowson, 1969).

The first successful calorimeter, using oxygen under pressure for the combustion of a substance in a closed vessel was devised by Berthelot in 1881. Subsequent development by Mchler in 1892, Atwater in 1899 and Parr 1912 served to improve the cost of the original Berthelot apparatus in mechanical details (Ranjhan and Krishna, 1980).

Another major contributor to the determination of Calorific value of foods is Rubber whose works were published in 1885 and 1901. He measured the heats of combustion of a number of different proteins, fats and carbohydrates in a bomb calorimeter and also studied the heat of combustion of urine passed by a dog, a man, a boy and a baby. He then realised that the heat of combustion of a protein in the bomb calorimeter was greater than its calorific value to the body because the body oxidises proteins only tu Urea, creatinine, Uric acid and other nitrogenous end products which are themselves capable of further oxidation (McCance and Widdowson, 1969).

Using the various conversion factors available, a lot has been done in assessing the calorific value of different food substances. McCance and Widdowson (1969) reported the calorific value of different food substance including these figures on nuts. In that report, they indicated that the kernels of Almonds, Brazilnuts, Peanuts and Walnuts have 598, 644, 603 and 549 kilocalories per 100g of the dried samples respectively. Oni (1979) calculated the calorific value of three fresh water fish species obtained from Zaria dam, and reported the values of 558.40, 500.60 and 490.71 kilocalories per 100g of dried Alestes nurse, Tilapia Zilli, and Synodontis Schall samples respectively and Itam, et al, (1983) also reported the values of 540 kilocalories per 100g of dried sample as the Calorific value of African Walnut - Coula edulis. Just recently Ega (1986) reported that the samples of whole cassava unpeeled, whole cocoyam unpeeled, Peeled cassava (tissue only), Peeled cocoyam (tissue only), Cassava peels only, cocoyam peels only and Corn (TZB) provided 377.05, 370.59, 384.49, 379.36, 343.76, 351.49 and 360.85 kilocalories of energy respectively per 100g of the dried samples.

CHAPTER THREE

RESEARCH PROCEDURES

3.1 Sample Collection and Preparation for Analysis

The seeds of Tetracarpidium conophorum were obtained from Zaria Railway station on 15 August 1987 at 5.30 pm, as fresh samples just arriving from the South Western part of the country. In consideration of the report that the samples do not store well, unless immediately heated to 100°C (Irvine, 1961) the seeds were immediately stored in a freezer at 0°C before the analysis commenced. The hard shells of the seed samples were later broken, removed and the kernels used for analysis. For raw samples, the kernels of the fresh seed samples were used for analysis. Nevertheless, a number of randomly collected seed samples were cooked by boiling with distilled water in a glass beaker for one hour before the hard shell covers were removed, and the kernels of the cooked seeds used for analysis as cooked sample. The kernels of a number of fresh seed samples were split and the inner cotyledon removed and used for analysis so as to form the raw cotyledon sample. In addition the raw cotyledon removed kernels were also used so as to form the fourth sample of analysis.

The chemical analysis of the four samples commenced with moisture content determination. This was carried out on the fresh samples obtained. All other determinations however were carried out on dried samples.

The samples subjected to amino acid analysis were first defated exhaustively by the soxhlet extraction procedure using ether as solvent. The defated samples were then dried at 50°C to constant weight before they were sent for the amino acid analysis.

3.2 Determination of Moisture Content

The most widely used method for determining moisture content for plant and vegetable materials is by drying the sample at 105°C to constant weight (Allen, 1974). However, this method is reported not suitable for materials of high sugar products and products containing glycerol or propylene glycol, as at such temperatures there results loses in volatile fatty acids and some sugars decompose at temperatures above 70°C (A.O.A.C., 1980, and Ranjhan and Krishna, 1980). This is why drying at lower temperatures in vacuo is often employed to avoid loses in components other than water (Crampton, 1959). For all of these cases, the important factor in thermal drying is the maintenance of a differential between the vapour pressure of the substance to be dried and that of the atmosphere of the drying chamber (Willets, 1951).

Water occurs in foods in three different ways. These include free-water (in the inter granular spaces and within the pores of the material absorbed water (on the surface of macromolecular colloids) and bound water (in combination with various substances). These terms however are relative as the true moisture content is not known, the conditions selected for moisture determination are arbitrary (Pomeranz and Meloan, 1978). True moisture is however determined by the Karl-fischer method, a reaction between Iodine and sulphur dioxide, the stoichiometry of which depends on the presence of water molecules in the sample (Allen, 1974).

It is in consideration of the problems attached to the routine moisture content determinations that two temperatures were employed for the analysis. The first was at 105°C to constant weight (Allen, 1974) and the other was at 60°C to constant weight (Ranjhan and Krishna, 1980), so as to see the possible differences that may arise.

3.2 Method of Determining Moisture Content

A known weight of the sample, ground with pestle and morter, was dried in an air circulation oven. As two separate determinations were made, one set of samples was dried at 105°C to constant weight while the other set of samples was dried at 60°C to constant weight. The loss in weight is reported as moisture content at the specified temperature. Six replicate determinations were made in each case.

In both methods the moisture content was calculated as

$$\text{Moisture (\%)} = \frac{\text{Loss in weight(g) on drying}}{\text{Initial weight(g) of fresh sample}} \times \frac{100}{1}$$

3.3 Determination of Ash Content

The term "ash" refers to the residue left after incinerating the oven-dried sample at 525°C in a muffle furnace (Allen, 1974). It is a measure of the mineral content of the food material.

The principal errors in ashing arise through loses resulting from the use of too high a temperature or time allowed is insufficient. However, 525°C is being used as a compromise (Allen, 1974).

3.3.1 Method of Determining Ash Content (A.O.A.C. 1980)

A known weight of the oven-dried sample was heated in a 50 cm^3 petridish at 100°C until water was expelled. A few drops of pure olive oil was added and then heated slowly over a flame until swelling stopped. The addition of olive oil served to minimise frothing and swelling (Jacobs, 1958). The dish was placed in a muffle furnace at about 525°C and left until white ash was obtained. The ash was then moistened with distilled water, dried on steam bath, then on hot plate and re-ashed at 525°C to constant weight.

The percentage ash content was calculated as

$$\text{Ash } (\%) = \frac{\text{weight of ash (g)}}{\text{weight of dry sample}} \times \frac{100}{1}$$

Six replicate determinations were made in each case.

3.4 Determination of Total Organic Matter

The organic matter content is a measure of the total organic chemical constituents present in the sample. Such constituents include fats, carbohydrates, proteins nucleic acids, vitamins and related compounds (Ranjhan and Krishna, 1980).

Although loss on ignition is often used as a guide to organic matter content, many workers consider it too unreliable due to the potential loses of volatile salts, structural water and ammonia during combustion (Allen, 1974). However, for proximate analysis, it is accepted to determine organic matter content on the basis of loss due to ashing of the biological material. The value of the organic matter is then found by substracting the ash content from the dry weight of the biological material (Ranjhan and Krishna, 1980).

3.5 Determination of Mineral Elements

The discovery of flame spectroscopic methods of analysis has facilitated the quick quantitative determination of many elements, particularly the alkaline and alkaline earth elements which were formerly difficult to determine, provided certain precautions are taken regarding interferences, flame conditions and the like. The development of flame techniques using emission spectrometry and more recently absorption spectrometry has made sensitive and highly specific, methods available to the analyst.

The principle of emission spectrometry is based on the fact that when atoms of an element are heated in a flame, some of the heat energy is absorbed by the atoms which subsequently become excited. On reverting to the ground state, some electromagnetic radiations are emitted by the atoms and the intensity of the emitted radiations, which are characteristic of each element, is principally governed by flame temperature, other atomic species present and the number of atoms of the element in the flame at any one instant. If operating conditions are kept constant, the intensity of radiations will be a measure of this number, a situation which forms the basis of qualitative flame photometry.

Absorption, spectrometry however, is based on the fact that absorption of radiations of certain wavelength by atoms of an element in the ground state, leads to their excitation. The extent of absorption is dependent on the number of those atoms in the ground state and can thus be used as a quantitative method of determining this number.

The greatest advantage of both spectrophotometric methods is that they provide a simple means of determining minute quantities of substances. The methods are also generally accepted to be suitable for routine quantitative determination of an element in a given medium but not for routine qualitative analysis (Pinta, 1975). These spectrophotometric methods are based on Beer-lamberts law which state that the logarithmic ratio of the intensity of the incident light (Io) to that of the transmitted light (I) is referred to as the Absorbance (A) or optical density and is dependent on the concentration (C) of the absorbing specie and on the length (b) of the path of light through the solution.

One necessary pre-requisite which has to be satisfied before a successful quantitative analysis of an element by either flame technique is that the element which could be present in different compounds, some organic and inorganic complexes, must be available in an experimentally determinable form. In any case and as applicable to plant materials, the organic matter is first detroyed by either chemical or thermal methods, and the solution of the analyte element prepared in its ionic form.

Of the numerous analytical methods available for preparing the plant materials for elemental analysis, the most widely used are those of dry ashing and wet acid digestion methods. The later is however preferred (Allen, 1974). Since there are different wet acid digestion methods in use, the mixed acid digestion method reported by Piper in 1950 is employed for the present analysis.

3.5.1 Method of Digesting the Seed Sample (Mixed Acid Digestion)

An accurately known quantity of the oven dried sample in the range of 0.20 to 0.50g was placed in a 100 cm^3 Kjeldahl digestion flask. Pre-standing in 5 cm^3 of Nitric acid (70%) was allowed as it was not certain whether the sample was rich in protein, fat or resinous substance (Allen, 1974). 1 cm^3 of 60% v/v perchloric acid was then added, then followed by the addition of 0.50 cm^3 of sulphuric acid (98% w/w). This mixture was swirled gently and then digested slowly at moderate heat increasing afterwards, in an efficient fume cupboard. Digestion was continued for 15 minutes after the appearance of white fumes. The solution was then cooled, filtered through No. 44 filter paper into 60 cm^3 volumetric flask and diluted to volume with distilled water. Blank digestion was carried out in the same way without the sample.

The resultant solution was used for the analysis of the elements considered in the present work.

To prepare the sample solution for the analysis of Iron, about 20 cm^3 of distilled water was added to the acid digest and boiled for 10 minutes before filtering into a 50 cm^3 volumetric flask. In the case of manganese, because a pink colour was noticed after acid digestion the diluted solution was boiled for 10 minutes before making to volume as recommended by Allen, (1974).

Sodium and Potassium were determined by flame emission spectrophotometry while cadmium, calcium, cobalt, copper, iron, magnesium, manganese and zinc were determined by flame atomic absorption spectrometry. Phosphorus on the other hand was determined by the molybdenum blue colorimetric method.

3.5.1.1 Preparation of Standards

Prior to the aspiration of any of the sample solutions prepared into the appropriate instrument, standard solutions of ions of the particular element were prepared accordingly, aspirated into the instruments and readings recorded and calibration curves plotted. For all the determinations, distilled water was treated in the same way as the sample solution to give reference solutions in each case.

3.5.1.1.1 Cadmium (Whiteside, 1975)

2.1g of cadmium nitrate ($Cd(NO_3)_2$), was dissolved in 250 cm^3 of distilled water, the volume diluted to a litre in a volumetric flask and stored in a polythene bottle. This gave a stock solution of 1000ppm Cd. A range of 0-1.0ppm standards were prepared from the stock solution and appropriate volumes of acid extractants added to match the sample solution. The concentrations were determined using Pye Unicam SP 1900

Atomic absorption spectrometer.

3.5.1.1.2 Calcium (Allen, 1974)

The instrument used was Pye Unicam SP 1900 Atomic absorption spectrometer. Exactly 2.5g of calcium carbonate which had earlier been oven dried at 105°C was weighed after cooling and dissolved in 200 cm^3 of distilled water containing 5 cm^3 of Hydrochloric acid (specific gravity = 1.18). The solution was warmed to drive off the carbon dioxide, cooled and diluted to 1 litre to give a 1000ppm calcium stock solution. The stock solution was stored in a polythene bottle. A range of 0-100ppm Ca standards was prepared from the stock solution and sufficient lanthanum nitrate solution added to bring the concentration of lanthanum to 400ppm in each case as in the sample solutions. 400ppm of lanthanum was added because (using oxy-acetylene flame) it releases 40ppm of calcium from the interference effects of up to 30ppm aluminium, 35ppm phosphorus and over 50ppm of Iron (Allen, 1974). In all the standards, sufficient HCl and H_2SO_4 were added to bring their concentration to 1% v/v.

3.5.1.1.3 Cobalt (Whiteside, 1975)

Exactly 4.04g of cobalt (II) chloride was dissolved in 200 cm^3 of distilled water. The volume was diluted to 1 litre in a volumetric flask with distilled water to give a stock solution of 1000ppm Co. The solution was stored in a polythene bottle. A range of 0-1ppm cobalt standards were prepared from the stock solution and appropriate volumes of acid extractants added to match the sample solutions. The concentrations were then determined using a Pye Unicam SP 1900 Atomic absorption spectrometer.

3.5.1.1.4 Copper (Allen, 1974)

About 0.40g of Copper sulphate pentahydrate ($CuSO_4.5H_2O$) was dissolved in 200 cm^3 of distilled water and the final solution diluted to a litre in a volumetric flask to give a 100ppm solution of Cu. Working standards in the range of 0-1ppm Cu were prepared from the stock solution and in each case appropriate volumes of the acid extractants added to match the sample solutions. The absorbance was measured with Pye Unicam SP 1900 Atomic absorption spectrometer.

3.5.1.1.5 Iron (Allen, 1974)

About 0.50g of ferric chloride hexahydrate was dissolved in 1000cm^3 of water to give 100ppm Fe and the stock solution stored in a darkened polythene bottle. A range of 0-2.00ppm Fe standards were prepared from the stock solution and appropriate volumes of acid extractants added to match the samples.

The concentrations of both the standards and the samples were read using Pye Unicam SP 1900 Atomic absorption spectrometer. Using the calibration curve plotted from the readings of the standards, the concentrations of iron in the sample solutions were determined.

3.5.1.1.6 Magnesium (Allen, 1974)

About 1.0g of magnesium sulphate heptahydrate ($MgSO_4.7H_2O$) was dissolved in water containing 1cm^3 of sulphuric acid (98% w/w) and diluted to a litre with distilled water to give a 100ppm Mg solution. A range of 0 to 3ppm magnesium standards were prepared from the stock solution. Sufficient lanthanum nitrate was added to bring the concentration of lanthanum ions to 400ppm in each of the standards and in the sample solutions. 400ppm of lanthanum was added because when present in 1% v/v sulphuric acid, it release 3ppm of magnesium from the interference effects

of at least 100ppm of Calcium, 3ppm of aluminium and 10ppm of phosphorus (Allen, 1974).

Sufficient quantity of Hydrochloric acid (specific gravity = 1.18) was added in each of the standards to bring its concentration to 1% v/v as to match the sample solutions. The concentrations were determined using Pye Unicam SP 1900 Atomic absorption spectrometer at a principal resonance line of 285.21 nm.

3.5.1.1.7 Manganese (Allen, 1974)

Exactly 0.40g of manganous sulphate tetrahydrate ($MnSO_4.4H_2O$) was dissolved in distilled water containing $1cm^3$ of sulphuric acid (98% w/w) and then diluted to a litre to give 1000ppm Mn. The stock solution was stored in a polythene bottle. A range of 0 to 3ppm Mn standards was prepared and in each case sufficient acid extractants were added to match the samples. The concentrations of both standards and sample solutions were determined with Pye Unicam SP 1900 Atomic absorption spectrometer.

3.5.1.1.8 Phosphorus (Allen, 1974)

About 0.44g of dry potassium dihydrogen phosphate (KH_2PO_4) was dissolved in $200cm^3$ of distilled water and diluted to a litre to give a stock solution of 100ppm P. The molybdenum reagent was prepared by dissolving 25.00g of Ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}.4H_2O$) in about $200cm^3$ of distilled water in a beaker. About $280cm^3$ of sulphuric acid (98% v/v) was added to $400cm^3$ of distilled water with cooling. The molybdate solution was then filtered into the acid mixture, mixed thoroughly and then made up to 1 litre. The reagent was stored in the dark.

The stannous chloride reagent was prepared by dissolving 0.50g of

stannous chloride dihydrate ($SnCl_2.2H_2O$) in 250cm^3 of 2% v/v HCl. Appropriate volumes of the stock solution of 100ppm P was pipetted into 50cm^3 of volumetric flasks to give a standard range of 0-1.0mg phosphorus. Equal volumes of acid digest were then added to match the sample solution. 10cm^3 of the sample solutions were then pipetted into 50cm^3 volumetric flasks and at this point all standards and samples solutions were treated in the same way. The flasks were diluted to two third full and 2cm^3 of each ammonium molybdate and stannous chloride reagents were sequentially added and the solutions diluted to volume and left for 30 minutes.

The optical density was then measured at 700nm with a spectronic 20 colorimeter. A calibration curve was prepared and the milligram of phosphorus in the sample aliquot deduced. Blank determinations were also carried out in the same way, but without samples, and appropriate subtractions made. The concentration of phosphorus was calculated as

$$P(\%) = \frac{C(mg) \times \text{solution volume } (cm^3)}{10 \times \text{aliquot } (cm^3) \times \text{sample weight}(g).}$$

where C = mg of P obtained from the graph.

3.5.1.1.9 Potassium (Allen, 1974)

A stock solution of 1000ppm K was prepared by dissolving 1.9g of dry KCl in water and making up the volume to one litre. A range of 0 - 100ppm K standards were prepared from the stock solution and in each case an appropriate amount of acid digest added to match the samples. The concentrations were read using a Corning EEL flame photometer with the potassium filter.

3.5.1.1.10 Sodium (Allen, 1974)

0.25g of previously dried sodium chloride was dissolved in distilled water and then made up to one litre to give 100ppm Na ions.

Working standards in the range of 0 - 5.0ppm sodium ions were prepared from the stock solution and in each case appropriate volumes of acid extractants added to match the samples. The concentrations were measured using a Corning EEL flame photometer with a sodium filter.

3.5.1.1.11 Zinc (Allen, 1974)

To prepare a 100ppm Zinc stock solution, 0.44g of zinc sulphate heptahydrate was disolved in distilled water and then made up to one litre. A range of 0 - 5ppm Zinc standards were prepared and in each case appropriate volumes of acid extractants added to match the samples. The concentrations were determined using a Pye Unicam SP 1900 Atomic absorption spectrometer.

3.6 Determination of Crude Fat

When fat is defined as the material soluble in certain organic solvents, the accurance of bound fat as lipoprotein poses problems. It is because of such problems that the term crude fat is often employed and is known as the ether extract obtained after exhaustive extraction of the sample with ether solvent (A.O.A.C., 1980). The ether extract however consists of most ether soluble substances in the food material such as simple fat-fatty acids, esters, compound fat, neutral fat, sterols/pseudo fat (vitamins A, D_2, D_3, E, K), carotene, free fatty acids and cholesterol (Ranjhan and Krishna, 1980).

To avoid falling into such complications, some workers prefer to just call it as "ether extract", but the term crude fat is accepted for proximate analysis (Allen, 1974). In any case, the ether extraction method is based on the principle that non-polar components of the sample are easily extracted into organic solvents which in this case is diethyl ether. Direct extraction however gives the proportion of free fat but

gives no idea of the particular fatty acid involved (Oyeleke, 1984).

Generally, there are two methods for determining crude fat from biological materials. The first employs the weight loss of the dried sample after the exhaustive soxhlet extraction with diethyl ether solvent as crude fat, while the second uses the weight of the extracted fat obtained after evaporating off the ether as crude fat. The second method is reported to be more accurate (Allen, 1974) and is employed in the present analysis.

3.6.1 Method of Determining Crude Fat Content

2g of the dried sample was taken into a 50 x 10mm soxhlet extraction thimble which has previously been dried at 50°C to constant weight. The thimble was then put into 50cm^3 capacity soxhlet extractor. 100cm^3 of diethyl ether was put into a previously cleaned, dried and weighed 250cm^3 round bottom onical flask and then tightly connected to the soxhlet extractor containing the thimble. A reflux condenser was connected to the soxhlet extractor and extraction continued for sixteen hours (A.O.A.C., 1980) using a heating mantle. After extraction, the flask was then left in warm water bath until the volume of the liquid reduced to about 20cm^3. The liquid in the flask was then evaporated to dryness and the flask dried at 50°C to constant weight (Ranjhan and Krishna, 1980). The flask was then cooled and weighed. The crude fat content was calculated as

$$\text{Crude fat (\%)} = \frac{\text{weight(g) of extracted fat}}{\text{weight(g) of sample taken}} \times \frac{100}{1}$$

Six replicate determinations were carried out in each case. Blank determinations were also carried out in the same way without the samples analysed.

3.7 Determination of Crude Protein

The biological significance of proteins to all living systems, can not be over emphasised. It has a duel function of being used - both as an important constituent of growth and reproduction and as a fuel for energy production.

It is possible to determine true protein value after extraction with a suitable solvent by either gravimetric or colorimatric methods, but the protein solution needs to be fairly free from interfering substances, a condition which is not always conveniently achieved with plant materials (Allen, 1974). For this reason, it is a common practice in agricultural work to determine total organic nitrogen and multiply it by an appropriate factor, which depends on the percentage of nitrogen in that category of proteins, so as to obtain what is termed the crude protein value.

The most widely used factor is 6.25 which is based on the assumption that plant proteins contain 16% of nitrogen (Haikewal, 1969). Although plant proteins invariably contain 16 - 20% of nitrogen, Haikewal (1969) reported that the value of 16% is much lower for seed proteins. To be more specific, the proteins of peanuts and Brazilnuts contain 18.32% of nitrogen, the proteins of almonds contain 19.31% and the proteins of other treenuts contain 18.87% thus giving the conversion factors of 5.46, 5.18 and 5.30 respectively (A.O.A.C., 1980). It is in this regard that the factor of 5.30 is employed for the present analysis.

In preparing the solution of the biological material for the estimation of total organic nitrogen, numerous methods were proposed, most popular of which are the Kjeldahl digestion method and the dry ashing method, but the former is most universally used and hence was employed for the present analysis. The analytical procedure of Kjeldahl

digestion method involves converting organic nitrogen to ammonium nitrogen by the action of concentrated sulphuric acid in the presence of copper sulphate as catalyst and potassium sulphate to help raise the digestion temperature. The classical method of estimating the resultant ammonium nitrogen is by distillation, although other colorimetric methods such as the Nesslers reagent method do exist.

For the present analysis, the classical Kjeldahl digestion distillation method and the colorimetric method of Nesslers reagent were employed so that the two results could be compared. In Kjeldahl digestion distillation method, the resultant ammonium nitrogen obtained after acid digestion was decomposed by the action of a strong base. The released ammonia was received by a standard solution of hydrochloric acid which is then back titrated with standard sodium hydroxide so as to know the exact quantity of ammonia received by the hydrochloric acid solution.

In Nesslers reagent method however, the resultant ammonium nitrogen was determined by the addition Nesslers reagent and the absorbance of the resulting complex determined at 425nm (A.O.A.C., 1975).

3.7.1 Kjeldahls Method (Ranjhan and Krishna, 1980)

0.80g of the dried sample was accurately weighed into a $50cm^3$ Kjeldahl flask. 5g of potassium sulphate and 0.5g of copper sulphate were mixed with the plant material in the flask. To the mixture was added about $20cm^3$ of sulphuric acid (98% w/w) and the mixture shaken slightly. The flask was placed in an inclined position on a heating mantle and heated below the boiling point of the mixture until frothing ceased. Heat was increased later until the acid boiled vigorously and

digestion continued until the solution became colourless. The contents of the flask were cooled and transferred quantitatively to a round bottom flask with about 200cm^3 of distilled water. A few pieces of anti bumping granules were then added. A sufficient quantity of concentrated sodium hydroxide solution was added to the side of the flask until the solution became alkaline.

A distillation apparatus was assembled making sure that the tip of the dip extended below the surface of the standard hydrochloric acid (0.1N) in the receiving flask. The contents of the round bottom flask were distilled until all the ammonia has passed over into the standard hydrochloric acid solution. The receiver tip and condenser were washed into the acid solution in the receiving flask. The excess hydrochloric acid solution was then titrated with standard sodium hydroxide solution. Blank determinations were carried out using all reagents in the same quantities but without the material tested. Six replicate determinations were made in each case.

The percentage by weight of the total nitrogen in the dried samples was calculated as

$$\text{Total nitrogen (\% w/w)} = \frac{0.014\ (B-A)M \times 100}{W}$$

where B = volume in cm^3 of the standard sodium hydroxide solution used to neutralise the acid in the blank determination.

A = Volume in cm^3 of the standard sodium hydroxide solution used to neutralise excess acid in the test with the material.

M = Molarity of the standard sodium hydroxide

W = weight (g) of the material used for the test and the figure of 0.014 refers to the fraction of $\frac{14}{1000}$.

3.7.2 Nesslers Reagent Method (A.O.A.C., 1975)

The plant materials was first digested by adding 2g of potassium sulphate and 0.5g of copper sulphate to 0.1g of the sample in a $50cm^3$ Kjeldahl flask. $20cm^3$ of sulphuric acid (98% w/w), was added to the mixture and then the solution digested on a heating mantle until it turned colourless. The flask was cooled, the contents transferred quantitatively into a litre volumetric flask and diluted to mark with distilled water. This gave a concentration of 100ppm of the plant material (Johnson, 1941).

To prepare a calibration curve for the determination of ammonium nitrogen in the prepared solution of the plant material, 0.10g of ammonium chloride crystals was weighed and dissolved in a litre of water to give 100ppm of stock solution. A range of 0.84ppm to 25ppm standards were prepared from the stock solution. At this point, $5.0cm^3$ of each concentration including the solution of the plant material were pipetted into different test tubes followed with $3cm^3$ of 4M sodium hydroxide, then $2cm^3$ of Nesslers reagent were added. The Nesslers reagent was prepared by dissolving 100g of mercuric iodide and 70.0g of potassium iodide in a small amount of water. This was then added slowly with stiring to a cold solution of 160.0g of sodium hydroxide in $500cm^3$ of water and then diluted to one litre.

15 minutes after the addition of the Nesslers reagent to the standard and test solutions in the test tubes, the absorbance was read at 425nm (A.O.A.C., 1975) with a Spectronic 20 instrument. The concentrations of the test solutions were determined from the calibration curve earlier prepared.

3.8 Determination of Total Carbohydrate

Ranjhan and Krishna (1980) reported that the carbohydrate portion of biological material can be categorised into two groups. The first refers to the nitrogen-free extract which is often called the soluble carbohydrate and the second is the crude fibre or the insoluble portion of the carbohydrate. The soluble carbohydrate consists of all water soluble compounds which, in addition to the soluble carbohydrate as the major constituent of the mixture, also includes vitamins, monosaccharides oligosaccharides and even polysaccharides (Ranjhan and Krishna, 1988). The function of the soluble carbohydrate is mainly metabolic in nature (Allen, 1974).

The insoluble carbohydrate (crude fibre) comprises mainly polysaccharides consisting of hemicellulose and cellulose compounds whose functions are mainly structural and storage (Oyeleke, 1984). The total carbohydrate however comprises both the soluble and the insoluble portions of the carbohydrate. It is calculated by the method of difference (Jacobs, 1958). In this case the sum of the crude protein and crude fat contents is subtracted from the total organic matter content of the biological material to give the value of the total carbohydrate and nucleic acid content of the food material.

3.9 Determination of Soluble Carbohydrate

As mentioned earlier, this fraction refers to the nitrogen-free extract and its function is mainly metabolic in nature, that is why it is sometimes referred to as the available carbohydrate. The fraction is obtained after extracting the food material exhaustively with hot water. Although the result obtained in this way is somewhat arbitrary since in addition to the simple sugars, some other hot water soluble substance are brought into solution. For proximate analysis however,

hot water is accepted to give useful results (Allen, 1974).

There are at present a number of multifarious colorimetric and titrimetric methods of determining soluble carbohydrate in solution, however most of them have been reported inadequate, as they are suitable only for some specific groups of the soluble carbohydrate (Allen, 1974). It is in consideration of these facts that the widely used colorimetric reagent (Anthrone reagent) which was reported by Dreywood in 1946 was employed for the present analysis.

3.9.1 Method of Determining Soluble Carbohydrate Content

A stock solution of 250ppm Glucose was first prepared by dissolving 0.250g of a previously dried D-glucose in a volume of distilled water and thereafter making up the final volume to one litre. Working standards in the range of 0-100ppm glucose were prepared from the stock solution.

The anthrone reagent was prepared carefully by adding 380cm^3 of sulphuric acid (98% w/w) to 165cm^3 of distilled water in a boiling flask and the solution kept cold while mixing. 0.5g each of anthrone and thiourea were dissolved in the solution using a magnetic stirrer. The prepared reagent was transferred to a dark bottle and kept at about 1°C.

25mg of the sample was put into 50cm^3 conical flask and about 15cm^3 of water added. A glass bubble was then placed on the neck of the flask and the solution simmered gently on a hot plate for two hours. The solution was allowed to cool and then filtered through No.44 Whatman filter paper into a 25cm^3 volumetric flask. The paper was washed and the solution diluted to mark with distilled water. A blank determination was carried out in the same way but without the sample.

At this point $2cm^3$ of each standard and sample solution were put into a set of labelled boiling tubes. $10cm^3$ of anthrone reagent was added to each tube and mixed while the tubes remained in an ice bath. The tubes were then placed in a can of boiling water in a darkened fume cupboard and boiled for 10 minutes. The tubes were then placed simultaneously in an ice bath and allowed to cool in the dark. The optical density was then measured at 625nm with a spectronic 20 instrument. The calibration curve was prepared from the readings of the standards and the concentrations in the sample solutions extracted calculated as

$$\text{Soluble carbohydrate (\%)} = \frac{c(mg) \times \text{extract volume } (cm^3)}{10 \text{ aliquot } (cm^3) \times \text{sample weight}(g)}$$

where c = mg glucose obtained from the curve.

3.10 Determination of Calorific Value

The evaluation of the nutritional value of food by its energy value is usually termed its physical score. The calorific value of foods which is sometimes called its energy value or physicological fuel value is usually expressed in calories. This estimation is usually carried out by combustion or burning of foodstuff in the presence of oxygen using a bomb adiabatic calorimeter (Allen, 1974). By this method, burning 1g each of carbohydrate, protein and fat yields 4, 4, and 9 kilocalories of energy respectively. These factors are called Atwater factors and are useful in estimating the energy values after the chemical analysis of foods (Pearson, 1970).

The Atwater factors were employed in calculating the calorific values of the present sample of analysis in combination with the weight % of carbohydrate proteins and fats in the dry sample.

3.11 Determination of Amino Acid Composition

Inspite of its obvious importance in nutritional studies, amino acid analysis may be regarded as the fundamental elemental investigation of

the protein chemist. This is because if proteins are compounds within the strictest definition of organic chemistry, then their amino acid composition must be constant. Moreover, Haikewal (1969), reported that the functional, chemical and physical properties of a protein must depend upon the amino acid radicals, which occur in the peptide chain.

A protein chemist is prompted to the amino acid analysis of his sample because the nutritive value of proteins in a given food material is not necessarily determined only by its quantity but also by its quality which refers to the amounts and types of essential amino acids it has. It is in this regard that proteins are categorised into two. The first group is called "the first class proteins" because this group contains an appreciable amount of all of the essential amino acids. The "second class" proteins however do not usually contain all of the essential amino acids or if they do, contain one or more in negligible amounts (Haikewal, 1969).

Haikewal (1969) also reported that deprivation of all the amino acids to man leads to loss of body proteins with resultant weight loss, anaemia, hypoproteinemia and general muscular wasting. Also under such a condition, the organism is more susceptible to infection and less capable of dealing with disease and in addition, the withdrawal of a single essential amino acid in man leads to loss of apetite and negative nitrogen balance.

According to Haikewal (1969), nausea, dizziness and hypersensitivity to sounds are particularly observed in lysine deficiency in man. Administration of arginine deficient diet leads to reduction in spermatozoa. Hepatic and Renal damage have been reported to result from methionine deficiency. This amino acid is also necessary for normal hiar growth.

On the other hand, Harper (1973) reported that large excess of some amino acids induces severe toxic reactions. Methionine is the least tolerated of the nutritionally important amino acids. Harper (1973) also reported that about three times the amounts of methionine required by young rats causes some growth retardation when the protein content of the diet is low and double this amount causes histopathologic changes in pancreatic liver and kidney cells and also iron deposition in the spleen. In any case it is essential to determine the amino acid content of food since this will reveal how much is needed to supplement our diets.

The determination of amino acid composition of a given sample is carried out with the help of the commercially available Amino Acid Analysers, most of which are based on the ion exchange method of chromatography, since the method is easily automated (Allen, 1974). The ion exchange method depends on the differential binding of the charged groups to the supporting mediums (the resins). The degree of separation of the amino acids is related to their respective pKa values. In addition, aliphatic and aromatic amino acids are adsorbed in varying degrees to the supporting medium and this property plays an important role in the separation.

Before the sample is fed to the amino acid analyser, it is first of all defated and hydrolysed. It is the protein hydrolysate which is subsequently subjected to the amino acid analysis.

3.11.1 Hydrolysis of Proteins

Prior to the amino acid analysis of a sample, the protein must first be hydrolysed to its constituent amino acids which occur in the peptide chain. Haikewal (1969) reported that for a pure protein, the amino acid composition of the solid phase should be constant and

identical with that of the dissolved protein with which the solid phase is in equilibrium. Despite the fact that the precision of analytical procedures for the determination of amino acids has increased, the limiting factor has become the extent to which the composition of the hydrolysate is a true reflection of the composition of the parent protein. This is because discrepancies do arise owing to the decomposition of certain labile amino acids during hydrolysis and the fact that some amino acids are liberated from the peptide linkage only with difficulty (Oni, 1979).

For the analysis of the amino acid composition of a given protein sample, different methods of protein hydrolysis have been reported.

(a) Acid hydrolysis: This process employs 6M hydrochloric acid for the hydrolysis of the protein chains. It is reported to liberate sixteen of the eighteen common amino acids.

Tryptophan however is completely destroyed whereas Asparagine and Glutamine are converted to aspartic and glutamic acids respectively. Cysteine and Cystine are partially destroyed (Haikewal, 1969). The destruction of tryptophan is prevented by using additional specific reagents, Matheson (1974) recommended the use of thioglucollic acid and P-toluene-4-sulphonic acid, both of which have been found to give large yields of tryptophan. Other hydrolysis methods involve the use of methane sulphonic acid (Simpson, et al, 1960). The methods however are interesting but too novel to be adequately assessed.

(b) Alkaline hydrolysis: Using sodium hydroxide or Barium hydroxide (Pon, et al, 1970), the peptide bond of the protein chain can be cleaved but only tryptophan is liberated while other amino acids are destroyed (Matheson, 1974).

(c) Performic acid oxidation: This is achieved using a mixture of 30% v/v hydrogen peroxide and 88% v/v of formic acid in the ratio of one to nine. It liberates cysteine, cystine and methionine during hydrolysis (Standford, 1963).

Of the three different hydrolysis methods presented above, the method of acid hydrolysis was employed in the present analysis. It is normally carried out in two different ways (i) the sealed tube vial system and (ii) the open flask reflux method. Although the latter is employed because it is easier from the operational point of view and also because the dissolved oxygen is easily removed (Oni, 1979), the sealed tube technique is applicable to food analysis in preference to the hydrolysis under reflux (Schram et al, 1953).

However, Hirs et al, (1954) and Smith et al, (1954) have reported that acid hydrolysis even under controlled conditions results in the loss, sometimes complete, of certain amino acids.

3.11.1.1 Acid Hydrolysis of the Protein Samples

100mg of the defated vegetable sample was weighed into 250cm^3 round bottom flask and about 25cm^3 of freshly prepared 6M HCl was added. After adding some anti-bumbing granules, the flask was fixed to a reflux apparatus and the solution boiled at 110°C for 24 hours. The flask was then removed and the solution dried by suction.

To the dried residue was added 25cm^3 of distilled water and the solution again evaporated to dryness. The sample was then taken in a 100cm^3 volumetric flask and the solution diluted to mark. The filterate was thereafter concentrated to 10cm^3 and the pH appropriately adjusted using citrate buffer (Moore and Stein, 1951).

3.11.2 Separation of Amino Acids

3.11.2.1 Loading of Samples into Cartridges

The empty cartridges were washed with 10cm^3 of 0.2N sodium hydroxide. This was done by first removing the filler plug and forcing the liquid into the cartridge using 1cm^3 plastic tuberculin syringe. The cartridge was then emptied by inverting and shaking to remove all the liquid. The same procedure was repeated with 1cm^3 of pH 2.0 buffer.

While the cartridge was emptied and its resin equilibrated at pH 2.0, the internal standard (norleucine), amino acid standard and sample hydrolysate were loaded in the following order.

(i) 10ul of internal standard was pipetted with an automatic pipette (2x50 ul) into the empty cleared cartridge.

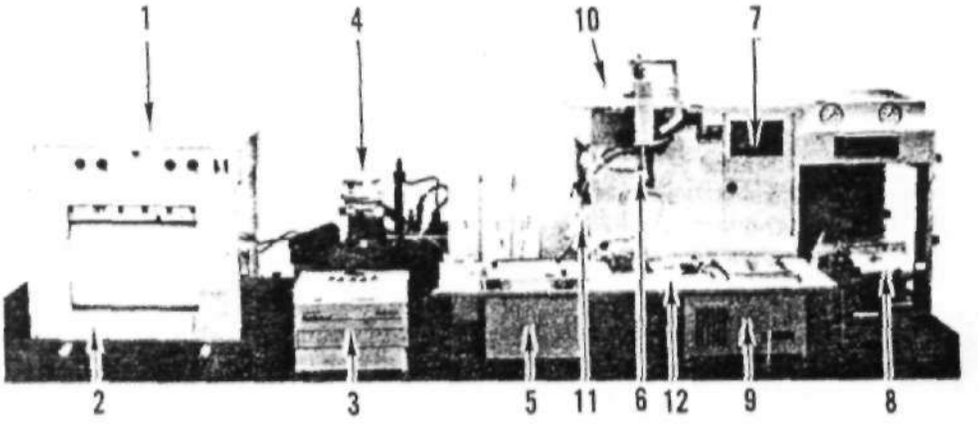
(ii) 10 ul of amino acid standard containing eighteen amino aci

(iii) 20 ul of hydrolysate in buffer of pH 2.0

Meanwhile a vacuum was applied while keeping the cartridge vertic and watched until the time when the loaded solutions were drawn into the filter. At this point the vacuum was removed. The filter plug was then replaced and the cartridge placed in the sample tray ready for analysis (Analyser system, 1973).

3.11.3 Chromatographic Analysis

The instrument employed for the analysis was the Technicon sequential Multisample Amino Acid Analyser (TSM) as shown in figure 3. This system was designed to separate, detect and quantify amino acids. It can separate and analyse free, acidic, neutral and basic amino acid from a protein hydrolysate in one and one quarter hours (Analyser system (1973).



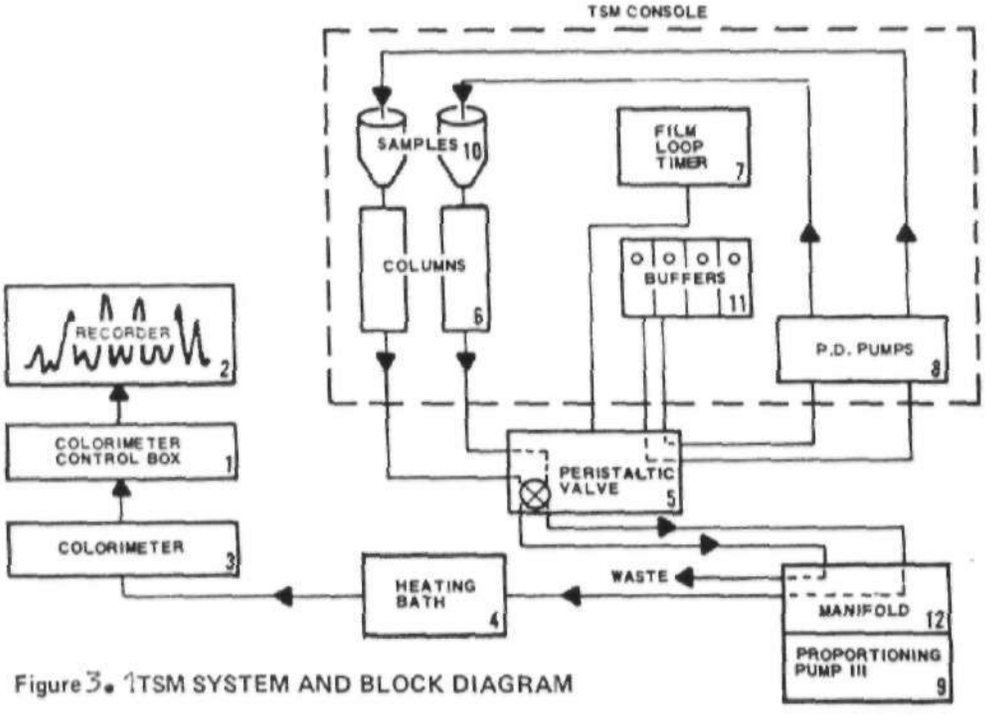


Figure 3. 1TSM SYSTEM AND BLOCK DIAGRAM

It comprises two columns, one 250cm^3 long for the neutral and acidic amino acids and the other 7.0cm long for the basic amino acids. Buffers are delivered to the columns through the sampler from two high pressure pumps. The sequence of flow of each buffer from the storage reserviors is controlled by a peristaltic valve and this valve also sequences the flow of the effluent from either column into the analytical system or the waste.

These column effluents that flow into the analytical system are mixed with a segmented stream of reagents introduced into the system through the propertioning pump (Figure 3.2). The combined streams then pass through a heating bath where the colours develop and the absorbance of the reaction stream is mentioned continously in a colorimeter and finally the signals from the colorimeter after amplification are traced on a two-pen recorder using a linear chart paper. A typical chromatogram is shown on figure 3.3.

The following amino acids were separated, detected and quantified by the aid of the standards.

Lysine	Threonine	Glycine	Isoleucine
Histidine	Serine	Alanine	Leucine
Arginine	Glutamic Acid	Valine	Tyrosine
Aspartic Acid	Proline	Methionine	Phenylalanine

3.11.3.1 Calculation of Quantity Percentage of Amino Acid in the Sample

Haikewal (1969) reported that the area under a peak is directly proportional to the product of the height of the peak and the width of the peak at half height. This forms the basis of the calculation of the concentration of each amino acid from the given peak area on the Chromatogram (Figure 3.4).

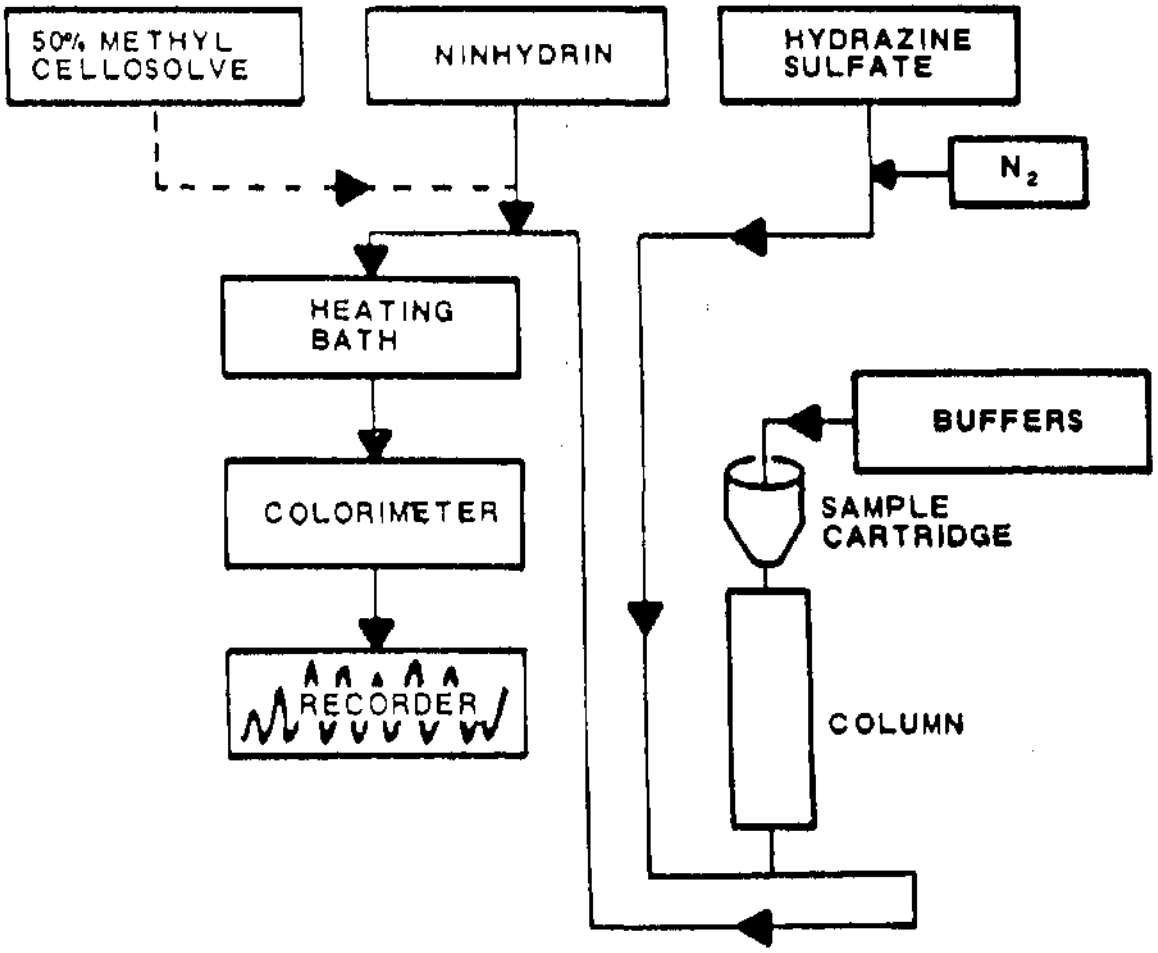


Fig. 3.2 A schematic diagram of steps involved in the AA analysis.

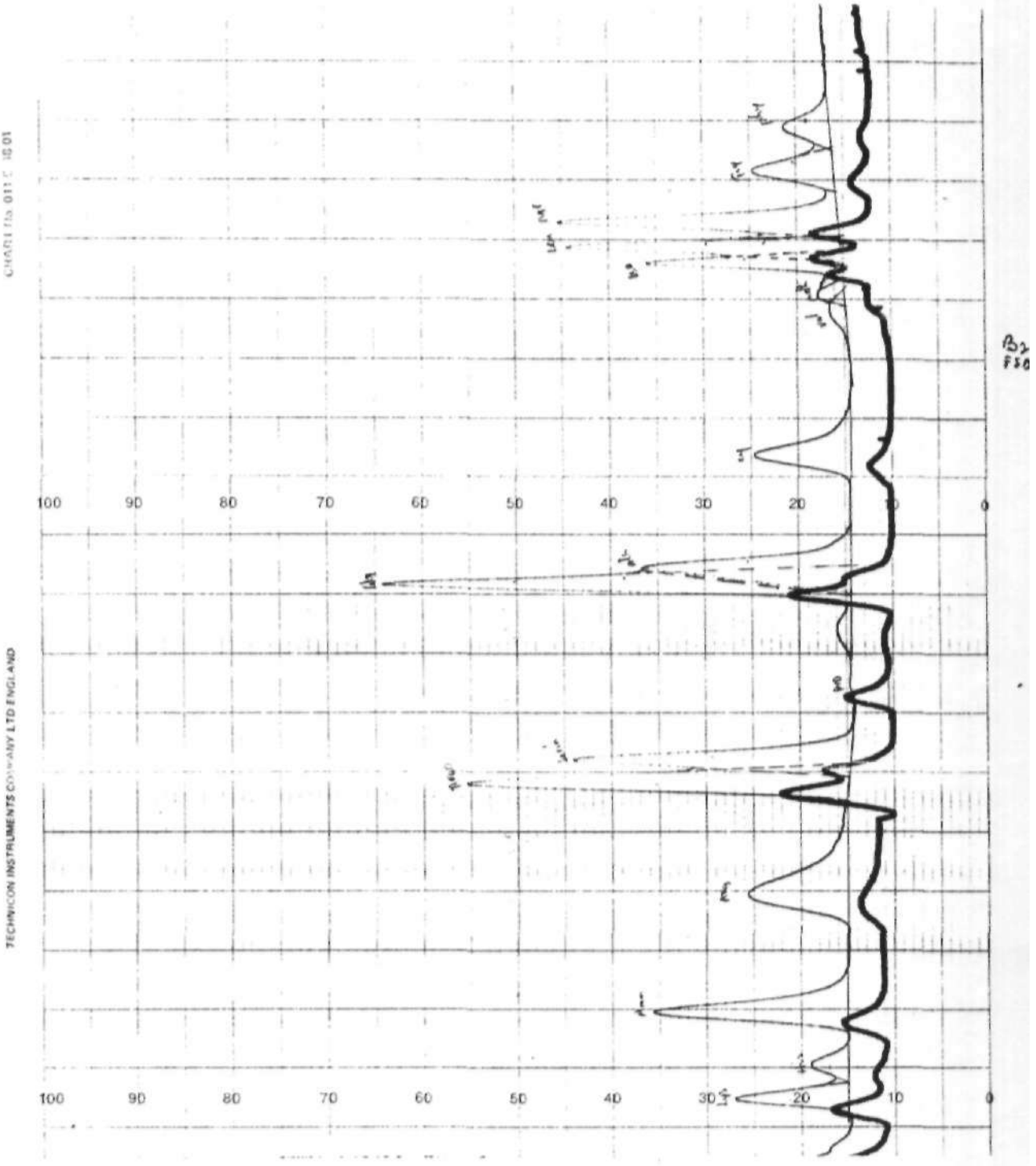


Fig. 3.3 A typical chromatogram of Amino Acid Analysis

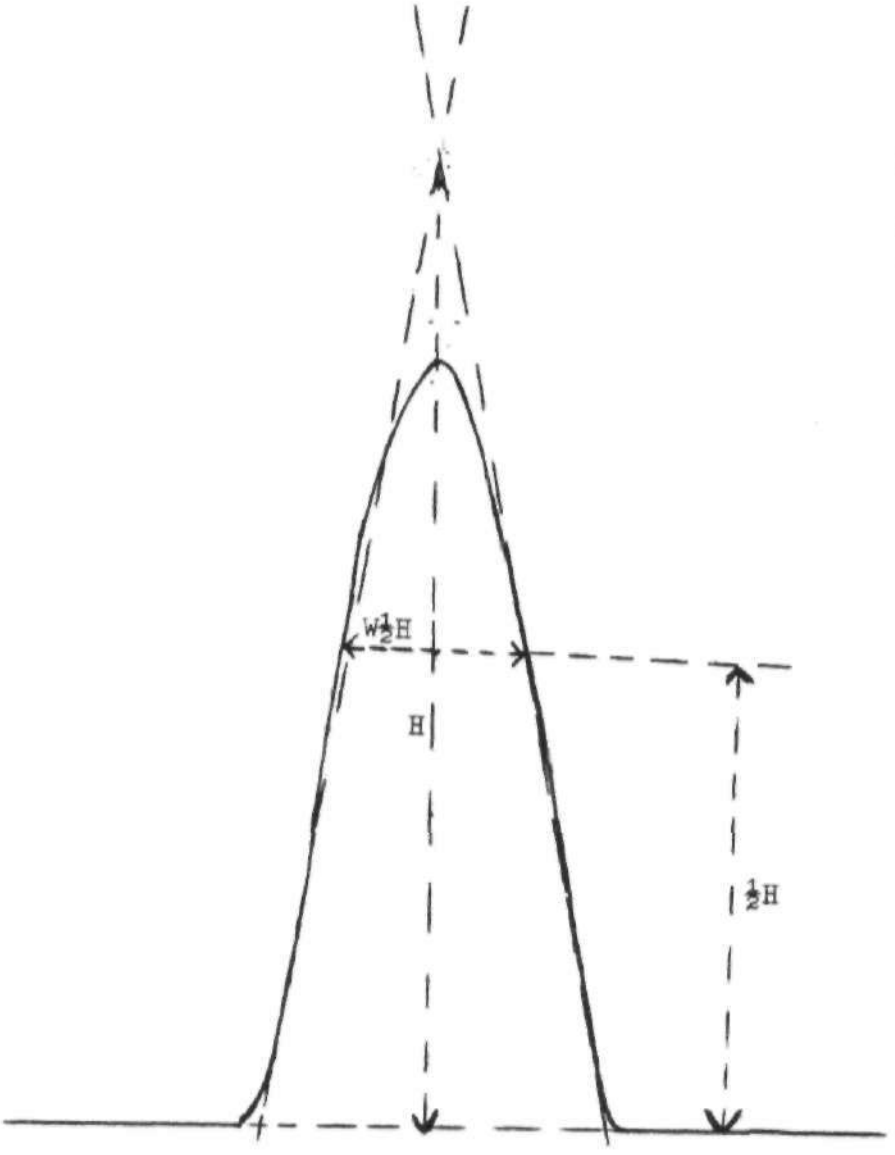


Fig. 3.4 Measurement of Peak height (H) Half height (½H) and Width at half height (W½H).

3.11.3.1.1 Method of Calculating the Concentration of Amino Acid

The net height of the peak is measured as H. The half height of the peak was located by dividing H by two. The width of the peak at half height was then measured with a ruler as $W\frac{1}{2}H$. The product of H and $W\frac{1}{2}H$ gives a figure which is representative of the area of the peak (Analyser system, 1973).

$$NE = \frac{\text{Area of norleucine peak}}{\text{Area of each amino acid}}$$

The amount of each amino acid present in the unknown sample was calculated using the following formula.

$$\text{Umoles} = \frac{Aup}{Aun} \times NE_{sp} \times UM_{sp}$$

where Aup = Area of unknown peak.

Aun = Norleucine area from the same chromatogram

NE_{sp} = Norleucine equivalent of the corresponding peak from a standard chromatogram

UM_{sp} = Umoles in the standard sample.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Moisture Content

The results of moisture content determinations carried out at 60°C on the fresh samples analysed are presented in tables 4.1 and 4.2 respectively.

Table 4.1 Moisture content at 60°C (on fresh weight basis)

Sample	Mean % (at 95% confidence limits
Raw	35.49 ± 0.81
Cooked	38.00 ± 0.78
Cotyledon	4.52 ± 0.16
Cotyledon-removed	36.64 ± 0.82

Where the number of determinations (n) = 6 in each case.

From the above table, the cooked whole sample (cooked sample) has the highest moisture content of 38%. This is to be expected, even though the seeds were cooked before the hardshells were removed, there is the possibility of water infusing through the hardshell cover into the seed kernel on the process of cooking, thereby increasing the moisture content. As the safe storage limit reported for plant food materials is 15% moisture (Ranjhan and Krishna, 1980) and 9% in the case of groundnut (Pearson, 1970), the result from table 4.1 signifies that only the raw cotyledon sample (Cotyledon sample) is suitable for storage purposes.

On application of T-test (Appendix 3), the difference between the mean % moisture contents of the raw whole sample (raw sample) and cooked sample is significant at 1% level. There is also a significant

difference at 0.1% level between the mean % moisture contents of the raw sample and the cotyledon sample. This suggests that the cotyledon has a significantly lower moisture content than the other part of the seed specie. The difference between the mean % moisture contents of the raw sample and the raw cotyledon-removed sample (cotylendon-removed sample) is however not significant at 5% level. This is also to be expected since the cotyledon sample has the least moisture content and it contributes a very small fraction of the weight of the seed kernel.

Table 4.2 Moisture content at 105°C (on fresh weight basis)

Sample	Mean % (at 95% confidence limits)
Raw	40.30 ± 0.87
Cooked	45.02 ± 0.56
Cotyledon	8.55 ± 0.74
Cotyledon-removed	42.23 ± 0.64

Where the number of determinations (n) = 6 in each case

As expected, the moisture content of all the samples at 105°C turns out to be higher than the corresponding values obtained at 60°C. This confirms the report by Allen (1974), A.O.A.C. (1980), Ranjhan and Krishn (1980) and Oyeleke, (1984), that in addition to water, some volatile compounds are lost at higher temperatures. In particular for values obtained at 105°C, they are only generally accepted to be those of moisture content, but actually they are the total volatile matter lost at this temperature (Jacobs, 1958).

The moisture content of the raw sample at 105°C differs markedly from the values of 23.50%, 4.50% and 4.70% reported for the raw samples of Walnuts, Peanuts and Almonds respectively by McCance and Widdowson (1

and with the value of 17.60% recently reported for African Walnut by Itam et al, (1983). These differences are however attributed to seasonal variations (James, 1952).

Table 4.2 refers to the mean % moisture contents of the raw sample. The values are significantly different at 1% level from those values obtained for the cooked sample. As obtained from table 4.1 the cooked sample also has the highest moisture content while the cotyledon sample recorded the least.

4.2 Ash Content

Table 4.3 refers to the ash content of the sample analysed.

Table 4.3 Ash content (on dry weight basis)

Sample	Mean % (at 95% confidence limits)
Raw	4.46 ± 0.04
Cooked	4.47 ± 0.04
Cotyledon	8.20 ± 0.71
Cotyledon-removed	4.25 ± 0.09

where the number of determinations(n) = 6 in each case.

The cotyledon sample has the highest value of ash content (8.20%) suggesting that it is potentially richer in mineral elements. There is no significent difference at 1% level between the mean % ash contents of the raw sample and the cooked sample. This implies that the extent of the effects caused, on the mineral content of the analyte sample as a result of cooking, are not significant enough to cause differences in the ash contents of the samples analysed. On the other hand, the slightly higher ash content of the cooked sample when compared to the

raw sample could be due to the possible infusion of some mineral elements from the hardshell cover to the seed kernel during the proce of cooking.

The difference between the mean % ash contents of the raw sample and the cotyledon-removed sample is significant at 1% level, and as expected, the cotyledon-removed sample recorded a lower value (4.25%) than the raw sample (4.46%) because the cotyledon has proven to have the highest value of ash content.

The ash content of the raw sample (4.46%) is much higher than the values for Almonds (3.0%), Brazilnuts (3.0%), Peanuts (2.40%) and Walnuts (1.40%) as reported by Pearson (1970). The value is however comparable to the value of 4.33% reported by Ega (1986) for the seeds of African locust bean.

4.3 Total Organic Matter Content

The results obtained from the total organic matter contents of the samples analysed are presented in table 4.4.

Table 4.4 Total organic matter content (on dry weight basis)

Sample	Mean % (at 95% confidence limits)
Raw	95.54 ± 0.04
Cooked	95.53 ± 0.04
Cotyledon	91.80 ± 0.71
Cotyledon-removed	95.75 ± 09

where the number of determinations (n) = 6 in each case.

On application of T-test to the results presented in table 4.4, no significant difference at 1% level is found between the mean % organic matter contents of the raw and cooked samples, thus implying that they have the same organic matter contents. There is however a

signigicant difference at 1% level between the mean % organic matter contents of the raw and the cotyledon samples, with the cotyledon samples recording a lower value. There is also a significant differe at 1% level between the mean % organic matter contents of the raw and the cotyledon-removed samples, reaffirming the earlier indication tha the cotyledon has a significantly lower organic matter content than other samples analysed.

In general, the result of the organic matter content of the samp analysed (table 4.4) follows the direct opposite trend exhibited by t ash content analysis (table 4.3). This is because the value of the organic matter content is calculated by substracting the ash content from the dry weight of the sample (Ranjhan and Krishna, 1980).

4.4 Mineral Content

Table 4.5 refers to the result of the mineral content analysis of the samples analysed. The analysis could not be extended to the r cotyledon sample because of the insufficient quantity of the sample available for analysis.

4.4.1 Cadmium Content

Table 4.5 contains information about the cadmium contents of th samples analysed. The raw and cotyledon-removed samples recorded the highest cadmium contents of 2.26 $ug.^{-1}$ On application of T-test, the is found no significant difference at 25% level between the mean % cadmium contents of the two samples. This implies that the cotyledon probably has the same cadmium contents as the cotyledon-removed sampl

There is however a significant difference at 5% level betwe n th mean % cadmium contents of the raw and the cooked samples. This

Element	Raw Sample	Cooked Sample	Cotyledon-removed sample
	Mean % (at 95% Confidence Limits)	Mean % (at 95% Confidence Limits)	Mean % (at 95% Confidence Limits)
Cadmium*	2.260 ± 0.580	1.010 ± 0.460	2.226 ± 0.580
Calcium	0.725 ± 0.030	0.681 ± 0.050	0.580 ± 0.050
Cobalt**	-	-	-
Copper*	20.110 ± 2.760	15.780 ± 2.760	9.110 ± 2.760
Iron	0.030 ± 0.010	0.030 ± 0.010	0.020 ± 0.005
Magnesium	0.106 ± 0.002	0.106 ± 0.002	0.110 ± 0.002
Manganese	0.010 ± 0.000	0.010 ± 0.003	0.010 ± 0.003
Phosphorus	0.860 ± 0.099	0.240 ± 0.099	0.600 ± 0.099
Potassium	1.640 ± 0.100	1.520 ± 0.099	1.520 ± 0.099
Sodium	0.076 ± 0.003	0.045 ± 0.003	0.036 ± 0.006
Zinc*	62.170 ± 1.430	75.070 ± 0.290	69.500 ± 2.150

Where the number of determinations (n) = 3 in each case

* = concentration in ugg^{-1}

** = element not detected

suggests that there was a significant loss of cadmium ions during the process of cooking the sample.

4.4.2 Calcium Content

From table 4.5, the calcium content of the raw sample is very high (0.725%) as compared with the values of 0.06%, 0.017% and 0.24% for Walnuts, Brazilnuts and Almonds respectively, as reported by McCance and Widdowson (1969). The value is also higher than the value of 0.11% reported for the kernels of Tamarindus seed by Ega (1986). From the result in table 4.5, the cooked sample has a calcium content of 0.681% and the cotyledon-removed sample (0.580%). This implies that some calcium ions were probably lost on the process of cooking and that the cotyledon probably has the highest concentration of calcium when compared to the other samples analysed. This is justified when consideration is made to the fact that calcium is essential for apical and root tip developments and also accumulates in the cell walls as calcium pectate (Allen, 1974) all of which are processes particularly important for the cotyledon which forms the food reserve of the young embryo (Corner, 1976).

On application of T-test, there is no significant difference at 5% level between the mean % calcium contents of the raw sample and the cooked sample, implying that the two have the same calcium contents and there is no significant loss of calcium during the process of cooking the sample. However, there is a significant difference at 1% level between the mean % calcium contents of the raw and the cotyledon-removed samples, suggesting that the cotyledon has a significantly higher calcium content than the other samples analysed.

4.4.3 Cobalt Content

Table 4.5 also includes the result of the cobalt determination of the samples analysed. The result shows that cobalt could not be detected in any of the samples analysed in the working concentration range employed. This was found to be so inspite of the large quantit (3.0g) of the sample used for the mixed acid digestion employed for t analysis. This implies that the concentration of the mineral element in that working range employed was too low to be detected. Neverthel cobalt is known to be necessary for enzyme activation in nitrogen fixation, but its importance to plant is yet to be fully understood (Allen, 1974).

4.4.4 Copper Content

The result of the determination of copper in the samples used fo this work has been presented in table 4.5. The raw sample recorded the highest copper content of 20.11 ugg^{-1}, followed by the cooked sam (15.78 ugg^{-1}). On application of T-test, there is found to be a significant difference at 5% level, between the mean % copper content of the raw and cotyledon-removed samples, an information which implie that the cotyledon probably has a higher copper content than the othe samples analysed. This is understandable if consideration is made to the fact that copper activates certain enzyme systems in plants espec those linked with oxidation processes (Allen, 1974) and these are som of the metabolic functions particularly required in the cotyledon whi forms the food reserve of the plant embryo (James, 1952).

The copper content of the raw sample (20.11 ugg^{-1}) of the specie analysed here is higher than the value of 11 ugg^{-1} reported for Brazil nuts by McCance and Widdowson (1969). It is also much higher than the value of 1.5 ugg^{-1} reported for African Walnut by Itam, et al, (1

4.4.5 Iron Content

From table 4.5, the raw and cooked samples have the value of 0.030%. This implies that there is no significant loss of iron during the cooking of the sample. The cotyledon removed sample however, has the least iron content of 0.020%, denoting that the concentration of iron might be higher in the cotyledon sample. This is understandable because iron occurs in the respiratory pigment, porphyrin, which is required in electron transfer (cytochrome) processes in plants and animals. It also activates some oxidases and is considered to be necessary for the chlorophyl synthesis (Allen, 1974). All these are particularly important for the cotyledon which is acting as a food reserve for the young embryo (James, 1955).

Despite the lower value of iron content in the cotyledon-removed sample, there appears to be no significant difference at 1% level between the mean % iron contents of the raw and cotyledon-removed samples, implying that the cotyledon does not have a significantly higher iron content than the other samples analysed.

The iron content of the raw sample (0.030%) is not much different from the values of 0.010% reported for Tamarindus seed kernel by Ega (1986) and 0.015% for African Walnut (Itam et al, 1983), but higher than the value of 0.001% reported for Almonds (McCance and Widdowson 1

4.4.6 Magnesium Content

From table 4.5, the mean % Magnesium contents of the raw and cooked samples are the same, thus implying that no loss of magnesium has occur during the process of cooking. The cotyledon-removed sample however ha the highest concentration of 0.110% thus suggesting that the cotyledon probably has the least concentration of magnesium ions. There is a significant difference at 5% level between the mean % magnesium content of the raw and cotyledon-removed samples, suggesting that the cotyledon has a significantly lower magnesium content than the samples analysed.

The magnesium content of the raw sample (0.106%) is lower than the value of 0.140% reported for African Walnut (Itam et al, 1983) and also lower than the value of 0.13% reported for Tamarindus seed kernel (Ega, 1986).

4.4.7 Manganese Content

As shown in table 4.5, both the raw and the cooked samples and even the cotyledon-removed samples have similar manganese content of 0.010%. This implies that there is no significant loss of manganese during cooking and that the cotyledon probably has the same manganese cotent as the other samples analysed.

On application of T-test, it is found that there is no significant difference at 1% level between the mean % manganese cotents of the raw and cooked samples and also between the former and the cotyledon-removed samples.

4.4.8 Phosphorus Content

Table 4.5 includes the results of the phosphorus contents of the samples analysed. The raw sample has the highest phosphorus content of 0.86%, then followed by the cotyledon-removed sample (0.600%) This

suggests that the cotyledon sample probably has the highest phosphorus content. This is justified by the report that phosphorus is particular important in the ADP-ATP cycle whereby energy is transferred between molecules in metabolic reactions (Allen, 1974) a phenomenon particularly important for the cotyledon which houses the embryo (James, 1952).

The cooked sample has the lowest phosphorus content (0.24%) thus implying that there is a significant loss of phosphorus during the cooking of the sample.

On application of T-test, to the results obtained, there is found a significant difference at 1% level between the mean % phosphorus content of the raw and the cooked samples, suggesting that there is a highly significant loss of phosphorus during the cooking of the samples. There is also a significant difference at 5% level between the mean % phosphor contents of the raw and the cotyledon-removed samples, thus implying th the cotyledon sample has a significantly higher phosphorus content than the other samples analysed.

The phosphorus content of the raw sample (0.860%) is much higher th the values reported for the African Walnut (0.339%) by Itam et al, (1983) and also higher than the values reported for Tamarindus seed kernel (0.300%) by Ega, (1986). The values are however close to that of 0.510 reported for Walnuts (McCance and Widdowson, 1969).

4.4.9 Potassium Content

As shown in table 4.5, the raw sample has the highest potassium content of 1.64%. The cooked sample has a lower value of 1.52%, implyin that there has been loss of potassium during the process of cooking the sample. The low potassium content of the cotyledon-removed sample suggests that the concentration of the mineral element may be higher in

the cotyledon sample. This is more understandable if consideration is given to the fact that potassium is essential to all plants for cell and membrane organisation and protein synthesis (Allen, 1974). These functions are undoubtedly essential in the storage organ of the plant enbryo, the cotyledon (James, 1952).

On application of T-test, there is found no significant difference at 5% level between the mean % potassium contents of the raw and the cooked samples and between the former and the cotyledon-removed sample. This signifies that there is no significant difference at 5% level between the potassium content of the cotyledon and the other samples analysed.

The potassium content of the raw sample (1.640%) is high and even higher than the values reported for African Walnut (0.612%) by Itam et (1983). It is also higher than the value of 1.00% reported for Parkia whole seeds by Ega (1986).

4.4.10 Sodium Content

Table 4.5 also includes the sodium content of the samples analysed. The raw sample has the highest sodium content of 0.076%, then followed by the cooked sample (0.045%) and the cotyledon-removed sample (0.036%). This information suggests that some loses of sodium ions have occured during the process of cooking and that the cotyledon probably has a higher sodium content than the other samples analysed. This could turn out to be so despite the fact that sodium mainly contributes to the ionic balance of the cell fluid and is essential for animals but apparently not for plants (Allen, 1974).

On application of T-test, the difference between the mean % sodium content of the raw sample and the cooked sample is significant at 1%

level implying that the loss of sodium during cooking is highly significant. The difference between the mean % sodium contents of the raw and cotyledon-removed samples is also significant at 1% level, denoting that the cotyledon probably has a significantly higher sodium content than the other samples analysed.

4.4.11 Zinc Content

The zinc content of the samples analysed is given in table 4.5. The raw sample has the least concentration of 62.17 ugg^{-1} while the cooked sample has the highest zinc content of 75.07 ugg^{-1}. This suggests that since the cooking was done before the hardshells were removed, there could have been some infusion of zinc ions from the hardshell cover into the seed kernel.

On application of T-test, the difference between the mean % zinc contents of the raw and cooked sample is significant at 1% level. The same applies to the difference between the mean % zinc contents of the raw and cotyledon-removed samples, thus suggesting that the cotyledon has a significantly lower concentration of Zinc than the other samples analysed. This may be so because zinc is regarded as an essential trace metal (Ajibola, 1984) and is mainly necessary for most organisms since it activates certain enzyme systems (Allen, 1974).

4.5 Crude Fat Content

The values of the crude fat content of the samples analysed are given in table 4.6

Table 4.6 Crude fat content (on dry weight basis)

Sample	Mean % (at 95% Confidence Limits
Raw	49.16 ± 2.03
Cooked	42.86 ± 2.34
Cotyledon	68.14 ± 1.57
Cotyledon-removed	52.32 ± 2.14

Where the number of determinations (n) = 6 in each case.

The crude fat content of the raw sample (49.16%) is found to be higher than the value for the cooked sample (42.86%). This implies that there has been some loses of crude fat during the process of cooking. This confirms the earlier assertion that some volatile fats and fatty acids are destroyed at temperatures above 70°C (Ranjhan and Krishna, 1980). The cotyledon sample has the highest crude fat content of 68.14%. This is evident even from a physical observation of the sample as it appeared oily.

On application of T-test, there is found a significant difference at 1% level between the mean % crude fat contents of the raw and the cooked samples. This implies that the loss of crude fat during cooking is significant at 1% level. There is also a significant difference at 1% level between the mean % crude fat contents of the raw and cotyledon samples. This also signifies that the cotyledon sample has a significant higher amount of crude fat than the other samples analysed. The difference between the mean % crude fat contents of the raw and cotyledon-removed samples is significant at 5% level but not at 1% level.

The crude fat content of the raw sample analysed (49.16%) is very close to the values reported for Peanuts (49.00%) and Walnuts (51.50%) by McCance and Widdowson, (1969). It is however higher than the value reported for African Walnut (31.10%) by Itam et al, (1983).

4.6.1 Crude Protein Content (Kjeldahls Method)

The results of the crude protein contents of the samples analysed by the classical Kjeldahls distillation method are given in table 4.7.

Table 4.7 Crude Protein Content - Kjeldahls method - (on dry weight basis).

Sample	Mean % (at 95% confidence limits)
Raw	20.62 ± 0.53
Cooked	23.37 ± 0.77
Cotyledon	11.42 ± 0.39
Cotyledon-removed	20.35 ± 0.31

Where the number of determinations (n) = 6 in each case.

It is clear that the raw sample has a lower crude protein value (20.62%) than the cooked sample (23.37%). On application of T-test, the difference is found to be significant at 0.1% level. One possible reason for this could be that, cooking the samples prior to analysis, helps in reducing some of the nitrate-nitrogen and nitrite-nitrogen (present in the sample) to ammonium-nitrogen which is subsequently determined as organic-nitrogen, thereby increasing the resultant crude protein value. However, the standard method reported of converting nitrate nitrogen to ammonium nitrogen is by adding salicylic acid at the digestion stage (Allen, 1974).

There is also a significant difference at 0.1% level between the mean % crude protein values of the raw and the cotyledon samples. This implies that the cotyledon has a significantly lower crude protein value than the samples analysed. There is however no significant difference at 5% level between the mean % crude protein contents of the raw and

the cotyledon-removed samples. This suggests that the two have the same crude protein values. This could be so because of the small fractional weight of the cotyledon as compared with the other part of the seed kernel.

The crude protein value of the raw sample (20.62%) is comparable to the value reported for Tamarindus seed kernel (19.20%) by Ega (1986) and also very close to the value reported for Almonds (20.50%) by McCance and Widdowson (1969). It is however higher than the value of 9.0% reported for African Walnut by Itam et al, (1983).

4.6.2. Crude Protein Content (Nesslers Reagent Method)

The results of the crude protein analysis by the Nesslers reagent method are given in table 4.8.

Table 4.8 Crude Protein - Nesslers reagent method - (dry weight basi

Sample	Mean % (at 95% confidence limits)
Raw	21.06 ± 0.26
Cooked	24.03 ± 0.40
Cotyledon	11.86 ± 0.13
Cotyledon-removed	20.84 ± 0.51

Where the number of determinations(n) = 6 in each case.

From the above table, the cooked sample has the highest crude protein content of 24.03%. As obtained by the Kjeldahls method, there is a significant difference at 0.1% level between the mean % crude protein values of the raw and the cooked samples. The difference between the mean % crude protein values of the raw and the cotyledon samples is

significant at 0.1% level. This agrees with the result obtained by the Kjeldahls method. Moreover, there is no significant difference at 2% level between the mean % crude protein values of the raw and cotyledon-removed samples. This also is in agreement with the result obtained by the Kjeldahls method.

4.6.3 Comparison of Kjeldahls and Nesslers Reagent Methods

A comparison of the results obtained on the crude protein values of the samples analysed by the two methods, shows that the values obtained by the Nesslers, reagent method (table 4.8) are consistently higher than those obtained by the Kjeldahls method (table 4.7). This is because a number of factors are thought to influence the Nesslers reagent method (A.O.A.C., 1975). These include:

(i) a number of volatile amines could cause turbidity with Nesslers reagent.

(ii) some volatile compounds such as certain ketones, aldehydes and alcohols might cause off colour on nesslerisation.

Owing to these problems, the Kjeldahls method is reported to be more accurate than the Nesslers reagent method and that the only condit in which the latter is more accurate than the former is when the concentration of ammonia is less than one milligram per litre (A.O.A.C., 1975) a situation which is very much lower than the ones obtained for our samples.

On application of T-test to the results obtained by the two method it is found that all the sample data gave results that are not significantly different at 5% level except the results from the cotyled sample which gave results that differ at 5% but not at 1% significance levels. This implies that the concentration of the interfering substan

in the cotyledon sample is high enough to affect the crude protein analysis by the Nesslers reagent method as compared to the same analysis by the Kjeldahls method.

4.7 Total Carbohydrate Content

Table 4.9 refers to the total carbohydrate content of the samples analysed.

Table 4.9 Total carbohydrate content (on dry weight basis).

Sample	% Total Carbohydrate
Raw	25.76
Cooked	29.30
Cotyledon	12.24
Cotyledon-removed	23.08

The cooked sample has the highest total carbohydrate content. This is due to the very low value of crude fat content, since the total carbohydrate is calculated by the difference method (Eka, 1971). In the same way, the cotyledon sample recorded the lowest total carbohydrate content because of its high crude fat content.

The total carbohydrate content for the raw sample (25.76%) is comparable to the value reported by Ega (1986) for Parkia seed kernel (24.79%) and slightly higher than the value reported by Pearson (1970) for Peanut (20.10%). However, the value is much lower than the value reported by Itam et al, (1983) for African Walnut (52.80%).

4.8 Soluble Carbohydrate Content

The results of the soluble carbohydrate analysis of the samples analysed are given in table 4.10.

Table 4.10 Soluble Carbohydrate Content (on dry weight basis)

Sample	Mean % (at 95% Confidence Limits)
Raw	5.78 ± 0.10
Cooked	3.87 ± 0.11
Cotyledon-removed	4.65 ± 0.12

Where the number of determinations (n) = 6 in each case.

From the above table, the raw sample has the highest soluble carbohydrate content of 5.78%. The cooked sample has a much lower value of 3.87%, an information which suggests that, although the seed samples were cooked before the hardshell covers were removed, there has been loses of soluble carbohydrate on the process of cooking. This is understandable if consideration is given to the fact that water was used for the cooking process and that the fraction under consideration is essentially water soluble. This implies that the hardshell cover has some degree of permeability which allows some contact between the water used for cooking and the seed kernel. The lower soluble carbohydrate value of the cotyledon-removed sample suggests that the cotyledon probably has a higher soluble carbohydrate content than the other sample analysed.

On application of T-test, there is found to be a significant difference at 0.1% level, between the mean % soluble carbohydrate contents of the raw and the cooked samples. This implies that the loss of soluble carbohydrate during the process of cooking is highly significant. There is also a significant difference at 0.1% level between the mean % soluble carbohydrate contents of the raw and the cotyledon-removed samples suggesting that the cotyledon probably has a significant higher amount of soluble carbohydrate than the other samples analysed.

The soluble carbohydrate content of the raw sample (5.78%) agrees closely with the value of 5.80% reported by Pearson (1970) for peanuts. This value for the raw sample is however lower than the value reported for cashew (8.10%).

4.9 Calorific Value

The results of the calorific value of the samples analysed are presented in table 4.11.

Table 4.11 Calorific value (Kilocalories per 100g of dried sample).

Sample	Crude Protein	Crude Fat	Total Carbohydrate	Total Calorific Value
Raw	442.44	82.48	103.04	627.96
Cooked	385.74	93.48	117.20	596.42
Cotyledon	613.26	45.68	48.96	707.90
Cotyledon-removed	470.88	81.40	93.34	645.62

The raw sample has a calorific value of 627.96 kilocalories per 100g, a value which is higher than that obtained for the cooked sample (596.42 kilocalories/100g). This confirms the earlier report that cooking the sample prior to consumption decreases the calorific value of the analyte sample (Akinrele et al, 1970).

The cotyledon sample has the highest calorific value compared to all the other samples analysed with a value of 707.90 kilocalories/100g. This value is expected because the cotyledon has higher crude fat content and that fat contributes the highest calories of energy than the other food constituents (Pearson, 1970).

In all the samples analysed, the contribution from fat to the total calorific value of the material is the highest and the protein contribution being the least. This is so because the contribution to the

total calorific value of any one food material, by each of protein, fat and carbohydrate depends on the quantity of each of these nutrients present in the analyte material.

The calorific value of the raw sample (627.96 kilocalories/100g) is lower than the value of 644 kilocalories/100g for Brazilnuts, comparable to the value of 603 kilocalories per 100g for peanuts and slightly higher than the value of 598 kilocalories/100g for Almonds as reported by McCance and Widdowson, (1969).

4.10 Amino Acid Composition

The results of the amino acid composition of the samples analysed are presented in table 4.12. The analysis could not be extended to the cotyledon sample because of the insufficient quantity of the sample available for analysis. Moreover, duplicate determinations were carried out on the raw and cooked samples, but only a single determination could be done for the cotyledon-removed sample. This is owing to a number of technical problems with the instrument employed.

From table 4.12, due to the low value of the number determination (n) in each case, the standard deviation values calculated did not make much statistical meanings to the determined mean values and have therefore not been considered. All the samples analysed however, have been found to be lacking on one essential amino acid (threonine). With the exception of aspartic acid the raw sample has a higher concentration of all the amino acids analysed than the cooked sample. However there is found no significant difference at 5% level between the mean % amino acid content of the raw and the cooked samples with the exception of valine which is completely missing in the cooked sample. This therefore implies that the amino acid content of the raw sample is not significantly higher than that of the cooked sample,

(g/16 g_N of dry matter, fat-free sample).

SAMPLE	Lysine	Histidine	Arginine	Aspartic Acid	Threonine	Serine	Glutamic Acid	Proline	Glycine	Alanine	Valine	Methionine	Isoleucine	Leucine	Tyrosine	Phenylalanine
RAW SAMPLE	2.95	3.17	8.51	4.86	-	5.19	15.20	4.93	10.65	3.68	0.38	1.06	4.40	4.88	4.17	2.77
COOKED SAMPLE	2.26	2.37	6.55	5.91	-	2.46	11.85	2.84	8.34	1.79	-	1.00	2.61	3.64	2.82	1.80
COTYLEDON REMOVED SAMPLE	0.75	1.42	2.19	2.41	-	2.62	2.46	2.04	15.02	2.67	-	0.99	0.60	2.10	3.30	-

The number of determinations (n) is 2 in the case of the raw and cooked samples and 1 in the case of the cotyledon-removed sample.

thus reaffirming the earlier report that cooking brings about reduction in the quantity of some amino acids, although actual destruction seldom occurs (McCance and Widdowson, 1969). In addition to reduction in the biological activity of the amino acids, cooking also appears to have lead to the destruction of valine which is also one of the indispensable amino acids.

The raw sample also has a nigher content of all the amino acids analysed (with the exception of glycine) than the cotyledon-removed sample. This therefore signifies that the cotyledon sample probably has a higher concentration of all the amino acids (with the exception of glycine) than the other samples analysed. This goes on to suggest that the cotyledon sample has a higher concentration of the indispensable amino acids than the other samples analysed. This is to be expected because of the role the cotyledon plays as a food storage organ of the young embryo (James, 1952). Moreover, the presence of valine in the raw sample and its subsequent absence in the cotyledon-removed sample suggests that the cotyledon probably contains valine.

A comparism of the essential amino acid composition of the raw seeds of Tetracarpidium conophorum with the FAO/WHO (1973) provision pattern and that of an egg reported by Itam et al, (1983), is given in table 4.13.

Table 4.13 Essential amino acid composition of the raw seeds of Tetracarpidium conophorum: comparism with FAO/WHO provisional pattern and Egg.

Amino Acid	g/16 g N- of dry		
	Seeds of Tetracarpidium conophorum	FAO/WHO	Egg
Isoleucine	4.40	4.00	6.60
Leucine	4.88	7.04	8.80
Lysine	2.95	5.14	6.60
Methionine + Cysteine	1.06	3.52	5.40
Phenylalanine + Tyrosine	6.95	6.08	10.80
Threonine	-	4.00	5.00
Tryptophan	-	0.96	1.70
Valine	0.38	4.96	7.40
Methonine	1.06	-	-

From the above table, it becomes clear that with the exception of isoleucine and phenylalanine + tyrosine, all the essential amino acids analysed in the raw sample are below the FAO/WHO (1973) recommended value. Tryptophan however could not be assessed because it has earlier been destroyed during acid hydrolysis. Threonine on the other hand is found to be completely absent.

On comparison of the estimated amounts of each of the indispensa mino acids required daily by human adults and children (Appendix 2) with the amounts of each of these amino acids present in the raw sample analysed as given in table 4.12, it is obvious that consumpti of 100.00g of the dried raw sample by human adults (70kg) daily will meet up the requirements for lysine, histidine, isoleucine, leucine and phenylalanine, whereas to meet up the same requirements for methionine and valine, up to 250 and 800g respectively of the dried

raw sample must have to be consumed daily. In addition, a child (12kg) needs to consume about 150g of the dried raw sample daily in order to meet up the requirements for phenylalanine, leucine, isoleucine, methionine and lysine.

The amino acid composition of the raw sample analysed is highly comparable with the values reported for African Walnut (Itam et al, 1983) as shown in Appendix 1. In those values for African Walnut, the following amino acids are found to have very similar contents with the analysed raw sample. These include histidine, arginine, serine and glutamic acid. The lysine, aspartic acid, valine, leucine and phenylalanine contents of the raw sample are lower than their corresponding values for African Walnut whereas the proline, glycine, methionine, isoleucine and tyrosine contents of the former are higher than the corresponding values of the latter. Threonine is found to be present in African Walnut but absent in the raw sample analysed, whereas alanine is present in the latter but lacking in the former.

4.11 CONCLUSIONS AND RECOMMENDATIONS

As earlier reported, nuts are nature's most concentrated foods and have a much higher nutritive value than any animal products except cheese (Itam et al, 1983). The seeds of Tetracarpidium conophorum are indeed rich in fats, proteins and essential elements and also have a high calorific value. In particular, the ratio of potassium to sodium (21.58) and that of zinc to cadmium (27.50) obtained for the raw seeds analysed, signify that the seeds are an excellent food material for hypertensive patients since sodium aids and potassium suppresses hypertention in some humans (George, 1973) and also cadmium aids and zinc suppresses hypertention in some humans (Schroeder, 1973). These seeds also serve as a good source of some essential amino acids at least at the recommended standard of FAO/WHO (1973).

It has been found in this study, as earlier reported by McCance and Widdowson (1969), that cooking the seed species, prior to consumption can be said to lead to a decrease in the amounts of some amino acids, although actual destruction seldom occurs. It also decreases the crude fat content, the soluble carbohydrate and the total calorific values, in addition to a decrease in the concentration of a number of essential nutrient elements. For instance, the ratios of potassium to sodium (21.58) and zinc to cadmium (27.50) for the raw seeds analysed, increased to 33.78 and 74.25 respectively on the process of cooking, thus reaffirming the earlier report by Eka (1982) that cooking decreases the concentration of some antinutritional substances. It is thus recommendable for the seeds to be consumed raw, where harmless.

The raw cotyledon sample has proven to have a higher concentration of most of the nutrients analysed than the other samples analysed, particularly crude fat, soluble carbohydrate, the essential nutrient

elements (as confirmed by its higher ash content) and most of the essential amino acids. This is certainly not unconnected with the function of the cotyledon which acts as a food reserve for the young embryo.

The crude protein analysis by the two methods of classical Kjeldahls distillation method and the colorimetric method of Nesslers reagent has helped to confirm that both methods are comparable for the present analysis inspite of the earlier report that the former is more accurate than the latter. The factors which make the colorimetric method to give results which differ slightly from the more accurate Kjeldahls method at the working concentration range employed, are probably not relevant for the sample analysed presently.

The fat and carbohydrate contents of the seeds of Tetracarpidium conophorum analysed are high and thus renders it useful for various feeding experiments aimed at assessing its biological value. Moreover, further analysis could be made on the mineral composition so as to have a broader knowledge of the concentration of various harmful elements possibly present and whether cooking has the effect of reducing the concentration of these elements.

On the other hand, its lipid could also be analysed to estimate the value of the essential fatty acids present. Another information of great nutritive significance, requiring further analysis is the Vitamin quantity and quality of the food material. Moreover, the possible use of the nuts in candies and in flavouring of dishes can also be considered. These suggested areas of research will give more comprehensive data for the seeds of Tetracarpidium conophorum.

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Amino acid	Arginine	Cy_2S_2	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophan	Tyrosine	Valine	Alanine	Aspartic acid	Glutamic acid	Glycine	Proline	Serine
Almond (A)	0.63	0.11	0.14	0.24	0.16	0.08	0.32	0.17	0.05	-	-	0.31	-	-	-	-	-	-
Brazilnut (A)	0.83	0.19	0.13	0.23	0.43	0.16	0.32	0.21	0.16	0.07	-	0.30	-	-	-	-	-	-
Peanut (A)	0.66	0.10	0.15	0.26	0.39	0.22	0.06	0.31	0.18	0.07	0.19	0.31	0.18	0.88	1.25	0.34	0.32	0.41
Walnut (A)	0.81	0.11	0.14	0.27	0.43	0.16	0.11	0.27	0.21	0.06	-	0.34	-	-	-	-	-	-
African Walnut (B)	8.25	-	3.23	3.97	7.59	5.88	0.98	3.95	3.79	-	3.24	3.97	-	8.71	15.12	4.24	4.40	4.96
Groundnut Meal (C)	12.3	1.14	3.04	3.58	7.09	3.90	0.91	5.60	3.04	1.24	4.34	4.27	4.19	11.82	21.12	6.30	5.06	5.53
Whole Cassava (D)	1.11	nd[x]	0.10	0.11	0.16	0.19	0.05	0.10	0.09	-	0.10	0.11	0.15	0.23	0.64	0.10	0.09	0.12
Whole Cocoyam (D)	1.03	0.21	0.34	0.38	0.87	0.62	0.10	0.52	0.49	-	0.46	0.53	0.49	1.40	1.12	0.57	0.36	0.62
African locust Bean (D)	2.04	2.24	1.16	1.45	2.52	2.58	1.00	1.66	1.26	-	1.08	1.51	1.72	3.31	5.14	1.48	1.63	1.78
Tamarindus Whole Seeds (D)	1.51	0.39	0.71	0.84	1.35	1.35	1.19	0.85	0.60	-	1.07	0.73	0.79	1.95	2.94	1.54	0.64	1.06

where A = MCance and Widdowson (1969) - Amino acid in g/1.0g of N
B = Itam, et al, (1983) - Amino acid in g/16g of N
C = Fetuga et al, (1973) - Amino acid in g/16g of N
D = Ega (1986) - Amino acid in % Dry matter, fat free basis.
nd = not detectable.

Appendix 2: Estimates of the amounts of each of the indispensable amino acids required by human adults and children (Harper, 1973).

Essential Amino Acids	Requirements for Human (g/day)		
	Male (70kg)	Female (58kg)	Child (1?kg)
Methionine	0.55	0.46	0.41
Tryptophan	0.16	0.13	0.17
Histidine	-	-	0.30
Phenylalanine	0.85	0.70	1.20
Leucine	0.88	0.72	1.20
Isoleucine	0.66	0.55	0.78
Valine	0.75	0.62	0.84
Lysine	0.66	0.55	0.96
Threonine	0.46	0.38	0.56
Protein	40.00	32.00	12.00

Appendix 3 T-TEST

The conditions necessary for any significance testing to operate are to:

(a) construct a mathematical model of the situation of which the hypothesis to be tested forms a part.

(b) state precisely the hypothesis to be tested,

(c) set up this hypothesis as null hypothesis (H_0) for the population, that is to say, set it up as being true. Any other hypothesis is the alternative hypothesis (H_1),

(d) define some measure of an unsatisfactory fit,

(e) find the probability with which (on hypothesis H_0), the observed value could be by chance, be equal to or more extreme than this measure,

(f) consider what evidence this probability provides against the hypothesis and what action should be taken next.

(1) The probability of 1 to 20 or 0.05, expressed as significant at 5% level is considered "significant".

(2) The probability of 1 to 100 or 0.01 expressed as significant at 1% level is considered "highly significant".

(3) Higher probabilities of 1 to 10 or 1 to 5 give levels which are considered not significant so that the sample data do not provide evidence to reject H_0 hypothesis.

(For a comparism involving any two sets of data, the appropriate expression for the t-distribution is given by

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$$

where $\bar{X}_1$ and $\bar{X}_2$ are the mean values of the first and second data, respectively.

S_1^2 and S_2^2 are the variances of the first and second data respectively.

n_1 and n_2 are the number of observations for the first and second data respectively.

(i) If the value of the t - calculated is less than the table t_f, α, (where f is the degree of freedom as n-1 and α is the probability), the null hypothesis is accepted and the two means are not significantly different.

(ii) If however the value of the t - calculated is greater than the table t_f, α, then the null hypothesis (H_o) is rejected and the two means are significantly different.